

ASSOCIATION OF FLOW-MEDIATED DILATION WITH SHEAR RATE IN PATIENTS AND HEALTHY VOLUNTEERS

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ABSTRACT

Introduction: Flow mediated-dilation (FMD) is a non-invasive test of endothelial function based on shear stress-dependent nitric oxide secretion causing vasodilation. Normalization of FMD to shear stress was previously proposed. The aim of present study is an examination of relationship between shear rate and FMD in patients with hypertension and atherosclerosis in comparison with healthy volunteers.

Methods: Three groups (11 with essential hypertension, 28 with advanced atherosclerosis and 26 controls) were examined. Flow mediated dilation after 5-minute cuff occlusion and post-deflation shear rate were assessed. Brachial artery diameters and blood velocities were obtained by means of high-resolution ultrasound machine.

Results: Positive correlations were found between FMD and shear rate with correlation coefficients depending on the disease. Statistical significance was found in atherosclerosis and control groups. Shear rate stimulus is most effective in healthy controls and least effective in hypertensive patients.

Conclusions: Post-ischemic shear rate or shear stress should also be taken into account while evaluating the endothelial function with vasodilation measurements.

KEY WORDS

Flow mediated dilation, shear rate, endothelial dysfunction, vasodilation, nitric oxide.

1. Introduction

Cardiovascular and civilization diseases such as hypertension, diabetes mellitus and atherosclerosis are associated with endothelial dysfunction [1]. The progression of these diseases leads to stiffening of arteries, where an abnormal nitric oxide secretion mechanism plays an important role [2].

An ultrasound technique assessing the brachial artery dilation due to reactive hyperemia is the international standard to assess endothelial function. Post-ischemic shear stress stimulates endothelium to release nitric oxide (NO) causing a dilation of a conduit artery. Flow-

mediated dilation (FMD) value provides an information about the endothelial function [3].

The goal of the present study is to compare shear rate-dependent vasodilation in two groups of patients with different cardiovascular disease progression with healthy volunteers.

2. Patients and Methods

2.1 Patients

A cohort of patients with essential hypertension and atherosclerosis, 18 to 85 year old, were recruited from the Department of Angiology, Second Chair of Internal Diseases, Collegium Medicum in Cracow over a one year period. Diagnoses were done previously by attending physicians. Healthy volunteers were recruited from the community and were matched to patients for age and body mass index (BMI). The local research ethics committee gave approval for the study. The investigations conformed to the principles outlined in the Declaration of Helsinki.

2.2 Measurements

Procedures consisted of height and weight recording, medical history and health habits questionnaires completion. Venous blood (10 ml) was taken. Levels of serum cholesterol, triglycerides, low-density lipoprotein (LDL), high-density lipoprotein (HDL) and serum creatinine were determined. Blood pressure was measured at the brachial artery using a validated oscillometric sphygmomanometer (Omron M3 Automatic Blood Pressure Monitor).

On the vascular testing day subjects were instructed to report to the laboratory between 7:30 and 11:00 AM after 12h fasting, abstained from caffeine, vitamin supplements and exercise. Tests were performed in supine position in a quiet, temperature-controlled (22-25°C) room after 20-minute rest to obtain a hemodynamic steady state. Heart

rate was monitored continuously using a three-lead ECG. A 14 x 50 cm automatic cuff (Endothelix, Vendys) was placed around the right forearm and ischemia was caused by a 5-minute inflation 40 mmHg over subject's systolic pressure. The ultrasound images of brachial artery were obtained longitudinally 2-12 mm above the antecubital fossa with high-resolution 14 MHz ultrasound transducer (Acuson S2000, Siemens). 10 images of brachial artery were acquired: 2 baseline and from 25th to 95th second after cuff deflation every 10 seconds. Each image was divided into 2D-mode and M-mode (Figure 1). Arterial diameters were measured from M-mode as an intima-to-intima distance at R-wave points. An open-source software (ImageJ, Wayne Rasband, National Institute of Health, USA) was used to evaluate the diameters. Mean blood velocities in the brachial artery were measured before the inflation and straight after deflation.

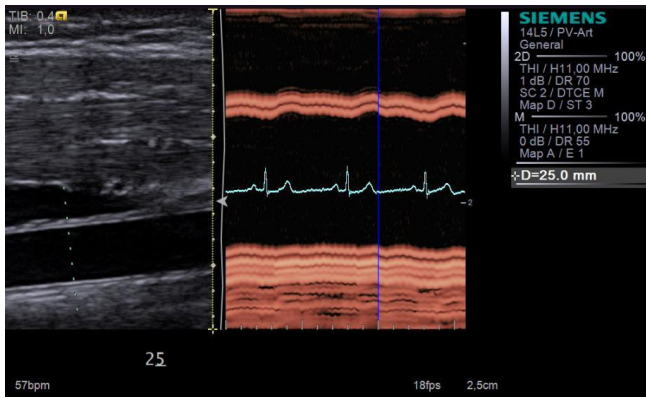


Figure 1. Ultrasound image of a brachial artery in B-mode (left) and M-mode (right)

2.3 Shear rate

Shear rate and blood viscosity contribute to vascular wall shear stress. Considering artery as a straight, cylindrical tube with rigid walls then shear rate (velocity gradient) will be a relation:

$$\dot{\gamma} = \frac{du}{dr},$$

where u is the blood velocity and r is the vessel radius. Poiseuille's law can be applied in vivo only after making assumptions that blood is an ideal Newtonian fluid with constant viscosity and the flow is steady and laminar. Shear rate can be determined [4]:

$$\dot{\gamma} = 4 \frac{u}{r}$$

Two values of shear rate were calculated: where u is the mean blood velocity after cuff deflation (SR1) and where u is the difference between mean velocities after and before cuff deflation (SR2).

2.4 Flow-mediated dilation

Flow mediated dilation is the proportional difference between the two diameters (after and before ischemia).

$$FMD = \frac{d_1 - d_0}{d_0} \cdot 100\%$$

where d_0 is the baseline brachial artery diameter and d_1 is the maximal value of brachial artery diameter in hyperemia (after cuff deflation).

2.5 Statistical analysis

All results are expressed as the mean \pm SD. Data were analyzed using the Statsoft, Inc. (2011), STATISTICA (data analysis software system), version 10. Pearson correlation analyses were used to determine relations among shear rate and flow mediated dilation. Group comparisons were made using analysis of variance. Bivariate and multiple regression analyses were performed to investigate the relationship between the parameters.

3. Figures and Tables

3.1 Patients characteristics

The clinical characteristics of 11 patients with essential hypertension, 28 patients with advanced atherosclerosis and 26 matched controls are summarized in Table 1. The groups were well matched for factors that may influence endothelial function such as age and body mass index.

Table 1. Baseline characteristics of patients

Characteristic	I Hypertension (n=11)	II Atherosclerosis (n=28)	III Controls (n=26)
Age, years	59 \pm 13	61 \pm 8	56 \pm 8
Weight, kg	75.6 \pm 13.5	79.0 \pm 14.5	73 \pm 13
BMI	26.3 \pm 3.4	26.5 \pm 4.0	25.2 \pm 3.2
Heart rate, min ⁻¹	61 \pm 8	66 \pm 11	63 \pm 9
SP, mmHg	141 \pm 10	138 \pm 18	128 \pm 13
DP, mmHg	83 \pm 7	76 \pm 10	76 \pm 7
PP, mmHg	58 \pm 8	62 \pm 15	52 \pm 9
Cholesterol level, mmol/l	5.0 \pm 1.2	4.5 \pm 1.2	5.5 \pm 1.3

LDL, mmol/l	3.4±1.3	2.9±1.0	3.5±1.2
Creatinine clearance, ml/min	92±26	109±26	97±22
ABI	1.16±0.08	0.78±0.23	1.01±0.26
IMT	0.674±0.166	0.801±0.161	0.640±0.076

3.1 Analysis and correlation

Differences in shear rate and flow-mediated dilation values between groups are shown in Table 2.

Table 2. Shear rate and FMD results

Param.	I Hypertension (n=11)	II Atherosclerosis (n=28)	III Controls (n=26)	P Ivs.III	P IIvs.III
Shear rate 1	1668±1047	1392±502	1922±509	0.325	<0.001
Shear rate 2	1351±931	1043±508	1581±465	0.320	<0.001
FMD	6.5±4.0	4.5±3.0	7.4±4.5	0.582	0.008

Values are mean ± SD. FMD=Flow-Mediated Dilation

FMD (%) was correlated with SR1 and SR2. In all groups FMD is positively associated with SR1 and SR2 (Table 3). The highest correlation coefficients are observed in healthy volunteers group, while the lowest in hypertension group. FMD (%) was correlated with SR1 and SR2. In all groups FMD is positively associated with SR1 and SR2 (Table 3). The highest correlation coefficients are observed in healthy volunteers group, while the lowest in hypertension group. Figures 2-7 present the correlations in each group.

Table 3. Pearson correlation coefficients

Relation		I Hypertension (n=11)	II Atherosclerosis (n=28)	III Controls (n=26)
FMD vs.SR1	R ²	0.288	0.299	0.447
	p	0.390	0.122	0.022
FMD vs. SR2	R ²	0.259	0.384	0.529
	p	0.443	0.044	0.005

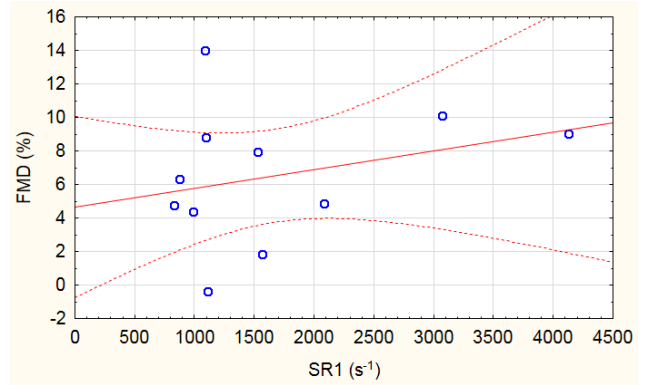


Figure 2. FMD vs. SR1 in essential hypertension group

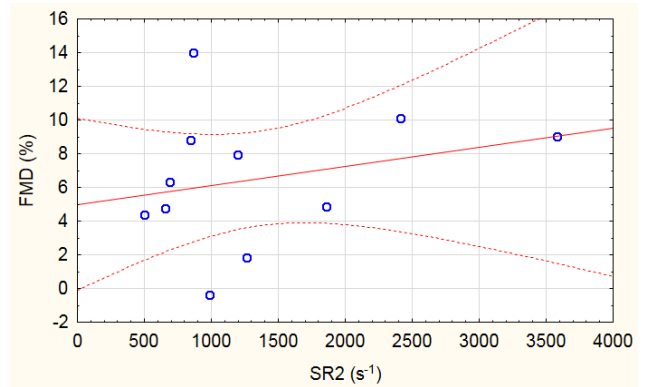


Figure 3. FMD vs. SR2 in essential hypertension group

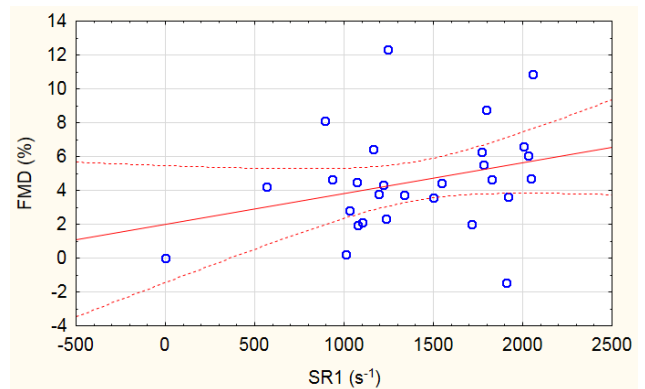


Figure 4. FMD vs. SR1 in atherosclerosis group

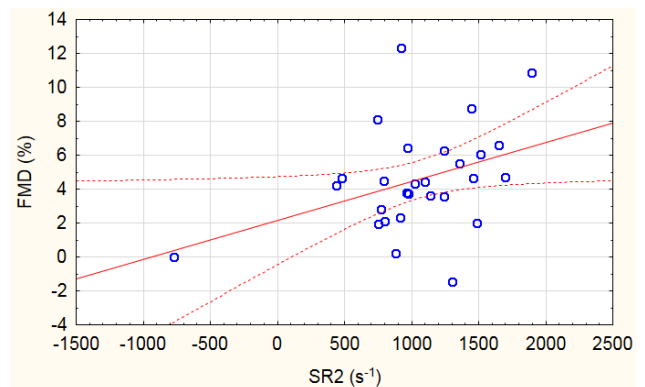


Figure 5. FMD vs. SR2 in atherosclerosis group

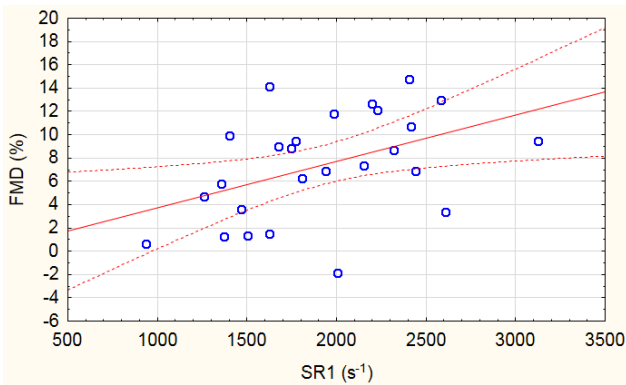


Figure 6. FMD vs. SR1 in control group

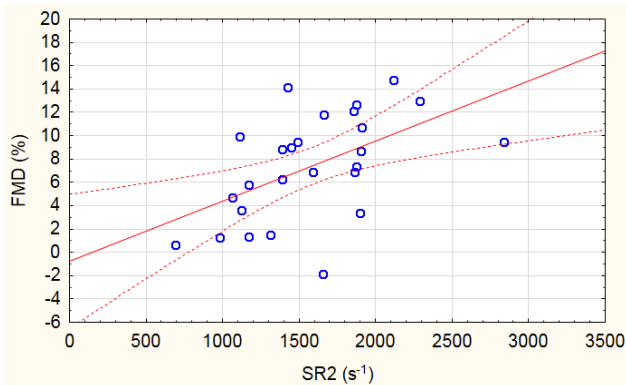


Figure 7. FMD vs. SR2 in control group

4. Discussion and Conclusion

As the hyperemic response varies among individuals [5], the FMD value may change not only due to endothelial dysfunction but also due to the stimulus. Normalization of FMD to shear stress was proposed previously [6, 7]. It was concluded that area under curve (AUC), not peak shear contributes to the FMD response. Limitation of this approach is that the shear stress and dilation have to be measured independently during two occlusions. Calculations of shear stress are more problematic as blood viscosities need to be obtained, though it was also suggested that the viscosity variability may reduce the association between shear and FMD and the viscosity evaluation may not be necessary [8].

The purpose of this study was to investigate whether peak shear rate leads to different FMD peak values, measured after single cuff occlusion, in 2 groups with different disease progression compared to control groups. Correlating FMD with SR2 tends to have higher coefficients with a higher level of significance than the correlation with SR1. This suggests that baseline shear rate also contributes to the stimulus and vessel dilation, not only its value after ischemia. Higher effect-to-stimuli ratios in advanced atherosclerotic group in comparison to

hypertensive group may be a consequence of more effective and efficient treatment. Due to a small number of patients with essential hypertension the results should be verified in further research.

Positive and significant correlation between FMD and shear rate indicates that FMD values should be corrected by stimulus. Our findings confirm that post-ischemic stimulus is most effective in healthy subjects and cardiovascular diseases significantly reduce the possibility of arteries to dilate.

Acknowledgement

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