The value of endothelium dependent vasodilatation in diagnosing coronary artery disease and its comparison with the results of routine diagnostic tests

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ABSTRACT

Objective: To determine the predictive value of flow-mediated vasodilation (FMD) compared with angina pectoris, exercise electrocardiography, and myocardial perfusion imaging (MPI).

Methods: This study was carried out in Shahid Madani Heart Center, Tabriz, Iran from April 2004 to September 2006. A total of 92 patients with chest pain syndrome were enrolled in this study. Using high resolution ultrasound system endothelial function was evaluated, and the result of the flow-mediated dilation (FMD%) was defined as the percent change in the internal diameter of the brachial artery during reactive hyperemia related to baseline.

Results: Coronary artery disease (CAD) was documented in 77 (83.7%) patients. The percentage of FMD was lower in patients with CAD compared with those without it (3.55 ± 3.71 versus 10.76 ± 4.61, p=0.001). In comparison with typical anginal chest pain (sensitivity 46.7%, specificity 80%), exercise stress test (sensitivity 75%, specificity 60%), and MPI (sensitivity 96.5, specificity 55.6%) the receiver operator characteristic curve showed the percentage FMD optimal cut-off value as ≤7.41 with a sensitivity of 87.0%, specificity of 66.7%, negative predictive value of 93.0%, and positive predictive value of 50%.

Conclusion: In patients with chest pain syndrome, the FMD is a sensitive indicator of CAD with moderate specificity that is unable to predict accurately the extent and severity of it.

Saudi Med J 2007; Vol. 28 (9): 1344:1349

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Received 2nd January 2007. Accepted 10th May 2007.

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The vascular endothelium is a monolayer of highly specialized cells that regulates the complex vascular milieu, acting as both a barrier to and facilitator of interactions between the plasma and the vessel. The endothelium accomplishes this goal through the expression of diverse molecules in response to a variety of mechanical and chemical stimuli. Of these factors, nitric oxide (NO) is essential.[1,2] In vessels stripped of endothelium, the response to stimulation by NO releasers such as acetylcholine or shear stress is lost, despite the persistent response to exogenous nitrates and sodium nitroprusside.[3] Thus, the dilation of vessels seen in response to these NO-releasing agents is designated as endothelium-dependent vasodilatation (EDV), whereas the response to exogenous nitrates is aptly called endothelium-independent vasodilation. The EDV has become synonymous with intact or normal endothelial function and preserved NO bioavailability. Assessment of endothelial function has long been limited by the invasive nature of measurements. In 1992 Celermajer et al[4] proposed a non-invasive method to assess endothelial function (flow-mediated dilatation [FMD]). The introduction of FMD opened the possibility of measuring endothelial function with minimal burden. Flow-mediated dilation was expected to be of use in risk stratification and in the assessment of the effectiveness of therapy.[5] Although numerous studies showed a relation between FMD and cardiovascular risk factors[6,7] and diseased or compromised patients,[8-11] evidence for the diagnostic value of FMD in obstructive coronary artery disease (CAD) is limited.[12-14] The purpose of the current prospective study was to estimate the potential usefulness of peripheral vascular reactivity noninvasively assessed by echocardiography in predicting angiographically assessed CAD in patients with typical or atypical angina and to compare its sensitivity and specificity with the results of exercise electrocardiography and myocardial perfusion imaging (MPI) in a subgroup of patients with suspected obstructive CAD and without recent myocardial infarction or previous revascularization procedures.

Methods. This study was carried out in Shahid Madani Heart Center, Tabriz, Iran from April 2004 to September 2006. A total of 92 patients who were scheduled for coronary angiography because of the clinical suspicion of CAD (typical or atypical angina pectoris and positive exercise electrocardiography or MPI) were enrolled in this prospective study. Typical chest pain was defined as retrosternal exertional chest pain with the dramatic response to rest or sublingual nitoglycerine, and atypical chest pain was defined as having 2 of these criteria. An exercise electrocardiography test (n=61) and MPI (n=38) were performed before hospital admission. Patients with prior history of coronary angiography, recent myocardial infarction (less than 3 month ago), surgical, or percutaneous revascularization, renal insufficiency, clinical heart failure, poor quality ultrasound images, and significant valvular heart disease were excluded from the study.

The cardiac risk factors considered in this study were defined as following: hypertension as either systolic or diastolic elevation of blood pressure above 140/90 mm Hg or ongoing antihypertensive medication, hyperlipidemia as a total serum cholesterol level >200 mg/dl and serum triglycerides >250 mg/dl or current treatment with lipid lowering drugs, smoking as active smoking within the past 12 months, diabetes mellitus as the use of oral hypoglycemic agents or insulin or having elevated fasting serum glucose levels (>140 mg/dL), positive familial history as having relatives of first degree with premature cardiovascular disease. The hospital's institutional review board approved the study and all patients gave their informed consent to participate in this study. Patients were prepared for the study according to the standard recommendations of Corretti et al.[15] They were instructed to fast overnight and to refrain from smoking or drinking coffee or tea for at least 8 hours before testing. Nitates were discontinued 24 hours before the test. The non-invasive determination of FMD was performed according to the standard protocol previously described in detailed.[4,13,15]

Ultrasound system (Vivid 7, GE, USA) with vascular software for 2 dimensional imaging (2D), color and spectral Doppler, a high frequency linear vascular transducer (10 MHz), with simultaneous electrocardiography monitoring system was used to acquire images with sufficient resolution for subsequent analysis. All images were saved in hard disk and VHS video tape to an offline post-procedure analysis. Patients were instructed to lie quietly, in supine position, for 10 minutes before the study. All the studies were performed in a temperature-controlled room (20°-25°C). In supine position with the arm in a comfortable position, the brachial artery imaging was carried out in a longitudinal plane above antecubital fossa. Scans of the brachial artery were obtained 10 cm proximal to the bifurcation. Measurements were carried out at rest, during reactive hyperemia (EDV), and after the sublingual administration of glycerol nitrate (endothelium-independent vasodilatation). After the optimal transducer positioning with the best scans and the clearest pictures of the anterior and posterior intimal layers, the transducer position was marked on the skin and the arm was kept in the same position during the entire procedure. At least 3 consecutive peak systolic frames were chosen and the average of the corresponding measurements was used as the indicator. A segment with sharp interface between lumen and vessel wall was selected for continues 2D imaging and Doppler study. The arterial diameter was measured from the anterior intima/lumen interface to the posterior intima/lumen interface as valuable anatomic markers at end-diastole seen as the beginning R wave on the continuously recorded electrocardiogram. All measurements were carried out at the same time in the cardiac cycle optimally achieved using ECG gating during image acquisition, peak of T wave was used to identify end systole and onset of the R wave was used as the marker of end diastole. A baseline rest image was acquired, and blood flow velocity was estimated by pulse Doppler from a midartery sample volume at peak systole; then arterial occlusion was created by cuff inflation to suprasystolic pressure. Typically, the cuff was inflated to at least 50 mm Hg above systolic pressure to occlude arterial inflow for a 5-minute period of time. Then the cuff was deflated and during the phase of increased shear stress and brachial artery dilatation, the longitudinal image of the artery was recorded continuously from 30 seconds before to 2 minutes after cuff deflation. A midartery pulsed Doppler signal also was obtained upon immediate cuff release and no later than 15 seconds after cuff deflation to assess hyperemic velocity. After 10 minutes of vessel recovery, resting scan, and measurements were repeated. Sublingual glyceryl trinitrate (0.4 mg) was successively administered to evaluate endothelium-independent vasodilatation. The last set of scans was performed 3 minutes after nitrate intake. Endothelium-dependent peripheral FMD was expressed as the percent change of brachial artery diameter 40-60 seconds after forearm occlusion release, with baseline resting diameter used as a reference. Endothelium-independent peripheral vasodilatation (induced by nitrate and so called, N-FMD) was expressed as the percent change of brachial artery diameter 3 minutes after sublingual nitrate administration, with baseline resting diameter used as a reference.

Coronary angiography, in multiple views, was performed the next day according to the standard Judkins method. At least 4 views (including 2 orthogonal views)

were acquired for the left and at least 2 orthogonal views for the right coronary arteries. The CAD was defined by the presence of angiographically detectable lesions of any severity and graded in 5 groups according to off-line quantitative angiographic analysis: group 0 = no vessel alterations, group 1 = vessel alterations with no lesions >50%, group 2 = significant lesions >50% in one major vessel, group 3 = significant lesions >50% in 2 major vessels, and group 4 = significant lesions >50% in 3 major vessels.

The data were analyzed using the Statistical Package for Social Sciences Version 13. Continuous measures were expressed as mean value ± SD. Discrete variables were described as counts and percentages. Continuous variables were analyzed according to the Student t test and Pearson correlation. Dichotomous variables were compared by Chi-square analysis. Calculations of sensitivity, specificity, and accuracy were performed according to standard definitions. A receiver operator characteristic curve (ROC) was generated to determine the predictive value of the percentage of FMD for significant coronary artery stenosis. A value of $p<0.05$ was considered statistically significant.

Results. The characteristics of the study population and ongoing therapy at the time of testing are displayed in **Table 1**. The mean age of our study group was 56.4± 10.2 and 55(59.8%) were male.

Coronary angiography. In 77 patients, the presence of CAD was established by coronary angiography, and in 15 patients it was excluded. The 6 (6.5%) had non-significant CAD, 24 (26.1%) had one vessel disease, 24 (26.1%) had 2 vessels disease, and 23 (25%) had 3 vessels disease.

Ultrasound study. Data obtained through ultrasound study were compared between those with and without CAD. As shown in **Table 2**, patients with normal coronary angiogram had significantly better endothelium dependent vasodilatation FMD in response to reactive hyperemia (10.76 ± 4.61% versus 3.55 ± 3.71%, $p<0.001$). Also they had a significantly better endothelium independent vasodilatation (with N-FMD = 10.40 6.05 versus 5.42 ± 5.44 = 0.008). The ratio of FMD/N-FMD was significantly higher in first group (1.49 ± 1.26 versus 0.50 ± 0.42, $p=0.03$). There was no statistically significant difference between other parameters, including absolute vessel diameters, and flows, in 2 groups. To obtain a cut-off value for FMD as a screen test for obstructive CAD a ROC analysis was conducted. The best cut-off point with the highest sensitivity (87%) and specificity (66.7%) to predict the presence of CAD was found to be at FMD ≤7.41% (**Figure 1**). To assess the value of FMD in discriminating 4 groups of CAD patients, mean value of FMD was compared. There was no significant difference between mean FMD values among CAD groups (**Figure 2**). Therefore, it is not possible to predict the severity of CAD based on FMD value

Typical chest pain was reported in 39 (42.4%) patients. Exercise stress test was carried out in 61 (66.3%) patients and MPI in 38 (41.3%) patients before admission. **Table 3** compares the sensitivity and specificity of FMD% with each of the former tests.

Discussion. The non-invasive evaluation of brachial artery FMD has merged as a useful tool to study vascular function. Celermajer et al[4] are the first

Table 1 - Characteristics of study population.

Parameter	No CAD n=15 (16.3%)	CAD n=77 (83.7%)	*P* value
Age	54.7 ± 10.8	56.7 ± 10.1	0.4
Male sex	3 (5.5)	52 (67.5)	0.001
Diabetes	2 (13.3)	22 (28.6)	0.1
Hyperlipidemia	4 (26.7)	41 (53.2)	0.06
Smoking	1 (6.7)	29 (37.7)	0.01
Hypertension	5 (33.3)	21 (27.2)	0.3
Aspirin	14 (93.3)	74 (96.1)	0.6
Beta blockers	12 (80)	69 (89.6)	0.3
Calcium blockers	4 (26.7)	20 (25.9)	0.8
ACE inhibitors	6 (40)	58 (75.3)	0.04

CAD - coronary artery disease, ACE - angiotensin converting enzyme

Table 2 - Results of ultrasound measurements.

Parameters	No CAD n=15 (16.3%)	CAD n=77 (83.7%)	*P* value
Baseline diameter (mm)	4.45 ± 0.63	4.61 ± 0.82	0.4
Hyperemic diameter mm)	4.64 ± 0.57	4.87 ± 0.98	0.2
FMD%	10.76 ± 4.61	3.55 ± 3.71	0.001
Post nitrate dimension	4.63 ± 0.53	4.87 ± 0.79	0.2
N- FMD	10.4 ± 6.05	5.42 ± 5.44	0.008
Baseline flow velocity	62.1 ± 18.9	62.49 ± 12.9	0.9
Hyperemic flow velocity	77.85 ± 20.9	68 ± 14.67	0.1
FMD / N- FMD	1.49 ± 1.26	0.5 ± 0.42	0.03

FMD - Flow mediated dilatation, CAD: coronary artery disease, N-FMD - post nitrate FMD

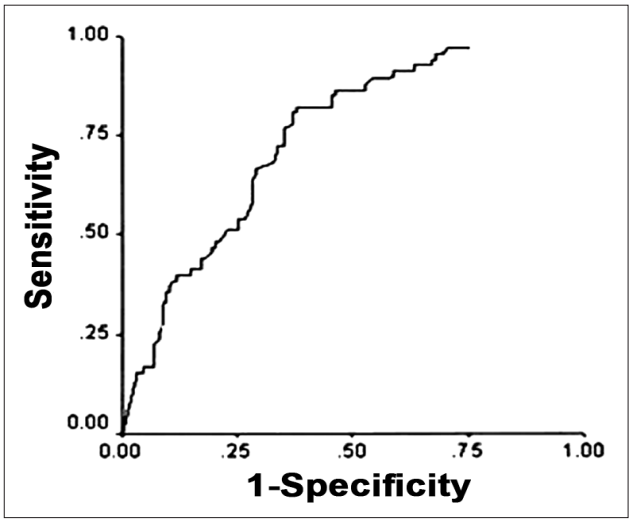

Figure 1 - Receiver operating curves (ROC) of flow-mediated dilatation (FMD%) in predicting coronary artery disease (CAD).

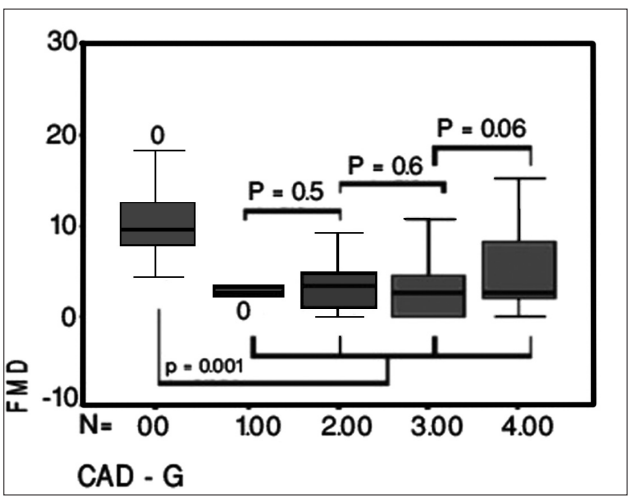

Figure 2 - Flow mediated dilatation among coronary artery disease groups.

Table 3 - Compares the sensitivity and specificity of typical chest pain, exercise stress test, myocardial perfusion imaging (MPI) and flow mediated dilatation (FMD%) in predicting coronary artery disease.

Test	Typical chest pain (n=39)	Exercise stress test (n=61)	MPI (n=38)	FMD%
Sensitivity (%)	46.7	75	96.5	87
Specificity (%)	80	60	55.6	66.7

to describe this technique, in which high-resolution ultrasonography will measure the brachial artery diameter at rest and following reactive hyperemia, induced by forearm cuff occlusion. The dilatory response associated with increased flow is thought to be endothelium dependent, and is subsequently used as a marker of endothelial function. Following the forearm occlusion a biphasic pattern is evident with an initial significant decrease in the diameter of the vessel as compared to the base diameters. After approximately 10 seconds the vessel diameter begins to increase, reaching peak at 40 seconds. Subsequently, the vessel diameter gradually returns toward the baseline value over the next 4 minutes. Ischemia is induced by inflation of a blood pressure cuff placed on the distal part of the arm, or on the proximal arm; the FMD is generally expressed as the percentage increase in brachial artery diameter after release of occlusion.[16] This dilation is mediated by endothelial NO release[2] in response to increased shear stress.[17] Thus, FMD is thought to reflect the endothelial NO-mediated regulation of vascular tone and diameter. Although numerous studies showed a relation between FMD and cardiovascular risk factors[6,7] and diseased or compromised patients,[8] evidence for the prognostic value of FMD was lacking until recently. Modena et al[18] showed that persistently decreased brachial endothelial function is a marker of a higher incidence of non-fatal cardiovascular events in hypertensive postmenopausal women (based on 32 events). Gokce et al[19] reported that brachial artery endothelial function predicts postoperative events in patients undergoing vascular surgery (based on 45 events). Surprisingly, a longer event-free survival was seen only in the upper tertile of FMD (>8.1%).[19] The brachial artery FMD appears to be a suitable surrogate for coronary arterial reactivity.[20] Takase et al[21] compared the brachial artery FMD to the coronary endothelial dysfunction measured angiographically after adenosine triphosphate infusion in 15 patients with suspected CAD. There was a strong correlation between abnormal coronary and brachial FMD (r = 0.78, $p<0.001$), implying that brachial artery FMD may be a noninvasive means of assessing coronary endothelial function.

Our test results for percentage FMD (10.76% ± 4.61% in control subjects versus 3.55 ± 3.71 in CAD group) are in agreement with the results reported by Celermajer et al[22] (8.2% ± 3.1% in control subjects versus 3.1% ± 2.7% in passive smokers) and Schroeder et al[14] (7.01% ± 3.51% for the no-CAD group and 3.73% ± 4.11% for the CAD group). However, our cutoff point for endothelial dysfunction at FMD% ≤7.4% is clearly higher than the one described by

Schroeder et al[14] (FMD% ≤4.5%) and very close to Neunteufl et al (FMD ≤10%).[8] This is probably a result of the use of different anatomic markers at the brachial artery.

In our study, we found a close relation between coronary artery disease and impaired flow mediated brachial artery dilatation. The FMD value of patients with CAD was significantly lower than those with normal coronary angiography. But, there was no significant difference between FMD value of groups with 1-3 vessel diseases. This means that endothelial dysfunction does not correlate with the severity of CAD. This is in contrast to the findings of Neunteufl et al,[8] but it should be emphasized that FMD is a functional parameter of early atherosclerosis, while CAD is an anatomical marker of advanced atherosclerosis in which FMD has been already compromised. Similarly, Jambrik et al[13] showed a weak negative correlation between FMD and angiographic Duke score.

Schroeder et al[14] reported a sensitivity of 71%, a specificity of 81% with a positive predictive value of 95% and a negative predictive value of 41% and at the study of Jambrik et al[13] the calculated parameters were 90, 37, 43, and 90%. In our study, a sensitivity of 87%, specificity of 66.7%, positive predictive value of 93%, and a negative predictive value of approximately 50% was obtained. This observed controversy in the specificity and positive predictive value of FMD may be related to patients with microvascular disease or early atherosclerosis without angiographically detectable plaques in these studies. For example, 129 of the 198 patients in the study of Jambrik et al[13] had normal coronary angiography findings. It seems that stress echocardiography may be a helpful test to differentiate patients with impaired endothelial function and without significant CAD from those with peripheral arterial endothelial dysfunction and obstructive CAD. Since impaired FMD is often associated with electrocardiographic[23,24] or scintigraphic[12] signs of ischemia during stress testing. On the contrary, wall motion abnormality during stress testing is unrelated to peripheral endothelial dysfunction[24] probably because echocardiographic positivity requires epicardial artery stenosis, and it is not evoked by pure microvascular abnormalities.[13]

Comparison with other screening tests. To determine the value of FMD% as an additional diagnostic test , we looked at the group of patients with documented CAD and negative stress test results (n=14). In this group, a FMD ≤7.4% was seen in 11 (78.6%) and we found that it may be a useful test with the additive diagnostic role in this subgroup of patients. Among 32 cases with a positive MPI result, a significant CAD was excluded in 4 patients with coronary angiography, a FMD of ≤7.4% was present in 2 of them and abnormality of both MPI and FMD in these patients may be due to microvascular disease or early atherosclerosis that disturbs endothelial function before the development of obstructive CAD. Also typical anginal chest pain was absent in 41 patients with documented CAD. An impaired FMD was seen in 36 (87.8%) of them again suggesting an additional diagnostic role for it.

In conclusion, the determination of impaired flow mediated dilatation in brachial artery with ultrasound is a non-invasive, non-radioactive dependent, and cost-effective approach with relatively high sensitivity and a moderate specificity. Combining the results of it with those of other traditional diagnostic tests may lead to more accurate non-invasive diagnosis of CAD and warrants larger and prospective studies.

Acknowledgments. *We are grateful to the Research Vice Chancellor of Tabriz University of Medical Sciences that supported this study.*

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