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Original Article

Correlation of endothelial dysfunction measured by flow-mediated vasodilatation to severity of coronary artery disease

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ABSTRACT

Objectives: Brachial artery ultrasound imaging during reactive hyperemia is widely used tool for quantifying endothelium dependent vasomotion. Angiodefender device is used for non invasive determination of percentage flow mediated vasodilation (FMD). An attempt is made to study whether endothelial dysfunction determined by FMD of brachial artery predicts the presence or absence of coronary artery disease and its correlation with the severity of coronary artery disease.

Methods: One hundred six patients admitted between May 2014 and April 2015 who were posted for coronary angiography diagnosed to have chronic stable angina on clinical basis and/or by exercise stress test, for evaluation of coronary artery disease were submitted to standard clinical evaluation, calculation of percentage FMD by Angiodefender device. Statistical significance of difference of categorical variables was tested using Fisher's exact test. Sensitivity, specificity, positive predictive value and negative predictive value of FMD were studied.

Results: There was no correlation between number of risk factors and percentage of FMD. Significantly higher proportion of cases with less FMD had higher prevalence of coronary artery disease and vice-versa. Significantly higher proportion of cases with positive stress test had less percentage of FMD and vice-versa. Significantly higher proportion of cases with less percentage of FMD and positive stress test had higher prevalence of obstructive coronary artery disease and vice-versa. Specificity was 100% when percentage of FMD was $\leq$10.

Conclusions: FMD an inexpensive and non-invasive test provides information regarding extent and severity of coronary artery disease.

1. Introduction

Non-invasive assessment of endothelial function has been proposed as possible inexpensive endpoint that could reflect cumulative cardiovascular burden and/or responsiveness to therapies at level of individual patients.[1,2] Of all methods that have been developed to assess endothelial function in human in vivo, most commonly employed is flow-mediated vasodilatation (FMD). FMD is expressed as vasodilatation induced in response to sudden increase in sheer stress and as such, quantifies capacity of endothelium to cause smooth muscle cell relaxation and vasodilatation when stimulated by release of nitric oxide.[3–5] FMD measured in forearm provides information which predicts extent and severity of coronary atherosclerosis, correlates with coronary endothelial function[6] and has prognostic implications that are similar to those of endothelial function measured invasively in coronary circulation.[7]

Impaired endothelium-dependent vasomotion (EDV) is a diffuse disease process resulting in abnormal regulation of blood vessel tone and loss of several atheroprotective effects of normal endothelium. Abnormal EDV can be detected in coronary circulation before development of angiographically significant atherosclerotic plaque and is associated with increased risk of future cardiac events. Although assessment of coronary artery endothelial function has advantage of examining vascular bed with greatest clinical relevance, it requires a specialized invasive procedure that is costly and not without risk.

Over the last 30 years, it has become clear that initiation and progression of atherosclerosis and its later activation to increase risk of morbid events, depends on profound dynamic changes in vascular biology.[8] Endothelium has emerged as the key regulator of vascular homeostasis. It not only has a barrier function but also acts as an active signal transducer for circulating influences that modify vessel wall phenotype.[9] Alteration in endothelial function precedes development of morphological atherosclerotic changes and

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can also contribute to lesion development and later clinical complications.[5]

In addition to coronary circulation, endothelial dysfunction occurs concurrently in peripheral arteries, and assessment of peripheral EDV provides an opportunity to evaluate larger patient populations in non-invasive fashion. Brachial artery ultrasound (BAUS) imaging during reactive hyperemia is widely used tool for quantifying EDV, angiodefender device too is used for non invasive determination of percentage FMD. Impaired peripheral endothelial function may also be a marker of increased future cardiovascular risk. BAUS can detect abnormalities in EDV in subjects at risk for atherosclerosis, and medical interventions and lifestyle changes that reduce coronary artery disease (CAD) risk are associated with improved peripheral endothelial function.[10–12]

Hence, an attempt is made to study whether assessment of endothelial dysfunction as determined by FMD of brachial artery predicts the presence or absence of CAD as diagnosed by coronary angiography, whether percentage FMD (%FMD) has any correlation with the severity of CAD and how does it correlate with traditional risk factors and whether FMD results have any additional value to exercise stress test in predicting presence and severity of CAD.

2. Methods

All patients of either sex admitted in Poona Hospital and Research Centre, Pune, Maharashtra between May 2014 and April 2015 with chronic stable angina and ready to participate were included in this observational study. Permission was obtained from institutional ethics committee and scientific advisory committee of the institution. Written informed consent of the patients was obtained after explaining details of the study, and risk involved. The study included patients of chronic stable angina diagnosed clinically undergoing coronary angiography. Exclusion criteria were patient undergoing coronary catheterization for acute coronary syndromes or for other reasons like for hypertensive crisis associated with troponin elevation, valvular heart disease, congenital heart disease, cardiomyopathy, or decompensated/severe heart failure, patients with baseline ECG abnormalities (pre-excitation syndromes, electronically paced rhythm, resting ST depression greater than 1 mm, and complete left bundle branch block). Based on previous study,[13] setting an alpha error at 0.05, and power at 80%, sample size of 95 patients was calculated by formula[14] for this study. We included 106 patients in the present study for better validation of results. Patients who were posted for coronary angiography diagnosed to have chronic stable angina on clinical basis and/or by exercise stress test, for evaluation of coronary artery disease were submitted to standard clinical evaluation, calculation of percentage FMD by Angiodefender device.

For purpose of this study chronic stable angina was defined as transsternal or retrosternal pressure or a choking sensation or pain that may radiate to left arm, jaw, neck, or back, brought on in predictable manner by exertion or by emotional upset. Sublingual nitroglycerin or cessation of exertion relieved discomfort.

Exercise test was performed in all patients before coronary angiography. ST segment depression during exercise defined as ischemic when $\geq$1 mm from the J point with flat or down sloping morphology and $\geq$1.5 mm from Y point with up sloping (slope of more than 1 mV/s) morphology, measured at least 60 to 80 ms after end of QRS complex were reported as positive stress test.

Cardiovascular risk factors such as male gender, body mass index (BMI), hypertension (systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg or being on antihypertensive medication), dyslipidemia (total serum cholesterol >200 mg/dl or taking lipid-lowering medication), diabetes mellitus (treated with oral hypoglycemic agent or insulin or having fasting glucose levels >126 mg/dl), family history of CAD (having first or second degree relatives with premature cardiovascular disease), and smoking (having smoked at least five cigarettes per day in last month) were noted in each subject.

Endothelial function was evaluated by using Angiodefender device (Everist) which is CE certified and has been proven to be equivalent to be gold standard BAUS imaging. This device uses hyperemia induced flow mediated dilatation mechanism for calculating FMD. Calcium channel blockers, nitrates and angiotensin-converting enzyme inhibitors were withdrawn 24 h before test and beta-blockers were discontinued 48 h before test. Variation in arterial diameter during rest and after reactive hyperemia was reported as %FMD, a variable that defines endothelial function. Nitroglycerine was administered as sublingual spray in dose of 0.4 mg and was used to evaluate endothelium-independent vasodilatation by direct action of drug on smooth muscle cells, thus excluding a possible confounding factor in evaluation of endothelial function. We have used hyperemia induced FMD for comparing the results. FMD (%) was categorized as <6.0%, 6.0–10.0% and >10%.[15]

Angiography was performed with a Shimadzu Bransist alexa system and routine projections were obtained for definition of coronary anatomy. Patients were divided into three groups according to presence and severity of atherosclerotic lesions: group 1: normal coronaries; group 2: coronaries with non-obstructive lesions (degree of stenosis <70%); group 3: obstructive CAD (degree of stenosis $\geq$70%) based on the opinion of three cardiologists.

Data on categorical variables is presented as percentage of cases. Data on continuous variables is presented as mean $\pm$ SD. Statistical significance of difference of categorical variables was tested using Fisher's exact test. Predictive significance of FMD and stress test in detecting CAD was tested using sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). P-values < 0.05 were considered to be statistically significant. Entire data was analyzed using Statistical Package for Social Sciences (SPSS) version 20.0, Inc. Chicago for MS Windows.

3. Results

Hundred and six patients (56 men and 50 women) who were enrolled in the study completed exercise stress test, FMD and coronary angiography. In all 17.0%, 44.3%, 30.2% and 8.5%, patients were between age of 40 and 49 years, 50 and 59 years, 60 and 69 years and 70 and 79 years respectively. Mean age of patients was 58.2 years with SD $\pm$ 8.3. In all 52.8% patients were males. In all 18.0%, 66.0% and 16.0% patients were normal weight (BMI 18.5–23), overweight (BMI 23.0–27.49) and obese (BMI > 27.5) respectively. Mean BMI was 24.9 $\pm$ 2.4 kg/m^2. Prevalence of diabetes, hypertension, and dyslipidemia was 46.2%, 47.2% and 22.6%, respectively. Smoking was prevalent only in male population (30.4%) whereas 46.2% had positive family history of CAD. Out of 106 cases studied, 74.5% had non-obstructive CAD, 16.0% had obstructive CAD whereas 9.5% were normal.

As depicted in Table 1, there was no statistically significant difference between number of risk factors and %FMD.

It can be seen from Table 2, %FMD was significantly associated with severity of coronary artery disease. Significantly higher proportion of patients with less FMD (<6.0%) had higher prevalence of ischemic heart disease (IHD) both obstructive and non-obstructive.

As shown in Table 3, outcome of stress test was significantly associated with distribution of %FMD. Significantly higher proportion of cases with positive stress test had less %FMD and vice-versa.

Table 1
Association between number of risk factors and FMD.

No. of Risk factors	FMD (%)								p value
	<6.0%		6.0–10.0%		>10.0%		Total		
	n	%	n	%	n	%	n	%	
<3	4	11.4	9	18.4	3	13.6	16	15.1	0.137
3–4	13	37.1	25	51.0	8	36.4	46	43.4	
5–6	15	42.9	15	30.6	11	50.0	41	38.7	
>6	3	8.6	0	0.0	0	0.0	3	2.8	
Total	35	100.0	49	100.0	22	100.0	106	100.0	

Table 2
Association between FMD and CAD.

FMD (%)	CAD								p value
	Normal		Non-Obstructive		Obstructive		Total		
	n	%	n	%	N	%	n	%	
<6.0	0	0.0	18	22.8	17	100.0	35	33.0	0.001
6.0–10.0	0	0.0	49	62.0	0	0.0	49	46.2	
>10.0	10	100.0	12	15.2	0	0.0	22	20.8	
Total	10	100.0	79	100.0	17	100.0	106	100.0	

Table 3
Association between stress test and FMD.

Stress Test	FMD (%)								p value
	<6.0%		6.0–10.0%		>10.0%		Total		
	n	%	n	%	n	%	n	%	
Negative	10	28.6	23	46.9	16	72.8	49	46.2	0.005
Positive	25	71.4	26	53.1	6	27.2	57	53.8	
Total	35	100.0	49	100.0	22	100.0	106	100.0	

FMD was studied in patients with positive stress test and was correlated with coronary angiography results. As depicted in Table 4, FMD result with positive stress test was significantly associated with coronary artery disease and distribution of severity of coronary artery disease. Significantly higher proportion of cases with less %FMD (<6.0%) and positive stress test had higher prevalence of obstructive CAD and vice-versa.

As shown in Table 5, when FMD was ≤10 specificity was 100% whereas sensitivity was between 37% and 51%.

4. Discussion

Present study compared endothelial function (% flow mediated dilatation) directly to coronary angiography for the presence and severity of CAD and also with exercise stress test, and relation of number of conventional risk factors with %FMD. Despite extensive studies and development of several risk prediction models, traditional risk factors fail to predict development of cardiovascular (CV) events in a large group of cases.[16–19] The extensive use of the most famous risk prediction model was proposed by the National Cholesterol Education Program III guidelines, in which the approach offered by the Framingham risk score to formulate a 10-year risk of CV events was embraced.[20] Today it is clear that the Framingham risk score as well as several other risk factor assessment models[21] have been shown to predict long-term outcome in a large population, but may not be able to predict short-term risk for individual persons, and cannot provide clear indications for cardiologists to identify, treat, and prevent near future victims of acute CV events in chronic stable angina patients.[22]

Table 5
Predictive values of FMD.

Parameters	Sensitivity	Specificity	PPV	NPV
FMD [<6.0%]	37	100	100	61
FMD [6.0–10.0%]	51	100	100	67
FMD [>10.0%]	13	0	55	0

Several studies reported that presence of conventional risk factors cause endothelial dysfunction and decrease in the FMD value.[1,23,24] In the present study it was found that worsening of% FMD did not correlate with the increase in number of risk factors. No statistically significant difference was detected in subjects with number of risk factors for the value of FMD. Mizia-Stec K et al[25] reported FMD was related only in low risk subjects and no correlation was found in patients with more number of risk factors. Similar findings were noted by Witte DR.[26] The present research substantiates the findings of above mentioned studies.

In present study, FMD was significantly lower (<6) in the individuals with obstructive CAD defined by coronary angiography. FMD values of <6 were good predictors of presence of coronary artery disease and definitely needs further evaluation of the disease with coronary angiography or other methods. Furthermore, FMD was an independent predictor of CAD. The results of the present study suggest that a patient with chronic stable angina and FMD in the range of 6–10% had coronary artery disease but all of them had non-obstructive disease. FMD > 10 reliably rules out obstructive coronary artery disease and FMD < 10 predicts presence of CAD. FMD < 6 predicts obstructive CAD and 6–10 suggests presence of CAD but non-obstructive.

Findings concerning the existence of a correlation between peripheral FMD and the extent of CAD were somewhat controversial. Corretti et al[27] reported no significant difference in FMD between patients with known CAD and a control group of healthy individuals. Their study concluded that morphological but not functional parameters of the brachial artery were associated with the extent of coronary artery stenosis and atherosclerotic wall changes in the carotid arteries in patients with severe CAD. Rohani et al[28] reported that morphological (i.e. brachial intima-media thickness) rather than functional (FMD) parameters provided information on the presence of CAD. Frick et al[29] concluded in their study that morphologic but not functional and mechanical

Table 4
Correlation between FMD and positive stress test with coronary angiography results.

FMD (%) and Positive Stress Test	CAD								p value
	Normal		Non-Obstructive		Obstructive		Total		
	n	%	n	%	n	%	n	%	
<6.0 (n = 25)	0	0.0	12	28.6	13	100.0	25	43.9	0.001
6.0–10.0 (n = 26)	0	0.0	26	61.9	0	0.0	26	45.6	
>10.0 (n = 6)	2	100.0	4	9.5	0	0.0	6	10.5	
Total	2	100.0	42	100.0	13	100.0	57	100.0	

parameters of the brachial artery were associated with the presence of CAD. Enderle et al[30] reported patients with clinically suspected coronary artery disease, %FMD discriminated between the presence or absence of CAD, but not with the severity, whereas intimal media thickness was associated more with the extent of coronary artery disease. Schroeder et al[31] reported that determination of endothelial dysfunction is a sensitive and specific screening test to predict the presence of CAD. Neunteufl T et al[32] stated that impaired FMD in CAD patients and non-CAD patients was related to the presence and extent of coronary disease. Their study stated existence of strong correlation between FMD and CAD severity.

In the present study, significantly higher proportion of cases with positive stress test had higher prevalence of CAD and vice-versa. FMD result with positive stress test was significantly associated with coronary artery disease and the distribution of severity of coronary artery disease. Significantly higher proportion of cases with less FMD (<6.0%) and positive stress test had higher prevalence of positive CAD with obstructive CAD status and vice-versa. It was also found that, subjects with stable angina and negative stress test but lower FMD values, had presence of CAD and also correlated with the severity of CAD on coronary angiography. Subjects with positive exercise test had low FMD values and vice versa.

In the present study, FMD <6 and FMD 6–10 demonstrated sensitivity of 37.0% and 51.0% respectively whereas specificity was 100% for the presence of angiographically significant coronary artery disease. This suggests that FMD value ≤10 could identify true negatives. Similar findings were noted by Simova I et al[33] in their study. Armin Arbab-Zadeh[34] reported sensitivity of 68% and specificity of 77%. A sensitivity of 71%, a specificity of 81% with a positive predictive value of 95%, and a negative predictive value of 41% was reported in the study conducted by Schroeder S et al.[31]

Mo Al-Qaisi et al[35] commented that brachial ultrasound FMD is useful in patients who have high risk of cardiovascular events. FMD detects silent cardiovascular risk and would be of help for early intervention in lifestyle changes as well as drug treatment. Dick et al[36] reported that FMD is a noninvasive and direct measure of artery function. FMD provides valuable and independent prognostic information. The authors opined that conducting and reporting FMD in a manner consistent with the physiology of the response to shear stress can improve the accuracy of FMD measurement. The present study also found that FMD value ≤10 would predict Coronary artery disease.

Limitations of the study were exercise myocardial perfusion imaging or intravascular ultrasound or fractional flow reserve was not performed. Many patients with significant coronary artery stenosis on coronary angiography might have physiologically insignificant lesion on testing with these modalities and vice vera. Novel risk factors, such as hyperhomocysteinemia, elevated C-reactive protein and endothelial independent vasomotion were not studied. The present research was conducted on a limited number of people. Further studies with larger sample size are recommended.

5. Conclusions

Significantly higher proportion of cases with less FMD had higher prevalence of positive CAD status and vice-versa. Significantly higher proportion of cases with positive stress test had less %FMD and vice-versa. Specificity was 100% when FMD was ≤10. There was no correlation between number of risk factors and% FMD.

Conflict of interest

Dr. Saurabh Sancheti, Dr. Prasad Shah, and Dr. Deepak Phalgune declare that they have no conflict of interest.

The manuscript has been read and approved by all the authors, that the requirements for authorship as stated earlier in this document have been met, and that each author believes that the manuscript represents honest work.

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