ORIGINAL RESEARCH PAPER

Cardiology

NON INVASIVE ASSESSMENT OF ENDOTHELIAL FUNCTION IN THE MANAGEMENT OF CORONARY ARTERY DISEASE

KEY WORDS:

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BACKGROUND

Atherosclerosis begins in childhood, progresses silently through a long preclinical stage, and eventually manifests clinically, usually from middle age. Over the last 30 years, it has become clear that the initiation and progression of disease, and its later activation to increase the risk of morbid events, depends on profound dynamic changes in vascular biology[**1**]. The endothelium has emerged as the key regulator of vascular homeostasis, in that it has not merely a barrier function but also acts as an active signal transducer for circulating influences that modify the vessel wall phenotype. Alteration in endothelial function precedes the development of morphological atherosclerotic changes and can also contribute to lesion development and later clinical complications[**2**].

The vascular endothelium is a large paracrine organ that secretes numerous factors regulating vascular tone, cell growth, platelet and leukocyte interactions and thrombogenicity.The endothelium senses and responds to a myriad of internal and external stimuli through complex cell membrane receptors and signal transduction mechanisms, leading to the synthesis and release of various vasoactive, thromboregulatory and growth factor substances. Endothelial dysfunction is thought to be an important factor in the development of atherosclerosis, hypertension, and heart failure.

A disturbance of endothelial function is considered as a key event in the development of atherosclerosis . Thus reliable assessment of endothelial function in humans appears highly desirable.With respect to the major endothelial functions, this aim can be achieved by different approaches:

1. measurement of morphological and mechanical characteristics of the vascular wall (intima media thickness, compliance, distensibility, and remodelling indexes);
2. determination of soluble endothelial markers (von Willebrandt factor, plasminogen activator, inhibitor complex thrombomodulin adhesion molecules, and *N*-oxides); and
3. measurement of the endothelium-dependent regulation of vascular tone at focal sites of the circulation.

The endothelium is of essential importance for the maintenance of vascular tone. It participates in the regulation of blood flow in response to changes in tissue and organ perfusion requirements.When blood flow increases through a vessel, the vessel dilates. This phenomenon has been coined flow-mediated dilatation (FMD).[**3**] Schretzenmayer was first to describe this physiological response, and FMD has been demonstrated subsequently in a number of conduit arteries in vitro and in vivo, in animals and in humans[**4**].

The effect of disease states and/or interventions on the blood flow response to cuff occlusion (reactive hyperemia) is underexplored. Current technology limits the utility of spectral Doppler to reproducibly assess changes in flow, which might provide useful information about endothelial function of the microvasculature. Tremendous interest exists in determining the clinical utility of brachial artery FMD. Investigators have hypothesized that endothelial function may serve as an integrating index of risk factor burden and genetic susceptibility, and that endothelial dysfunction will prove to be a preclinical marker of cardiovascular disease

Several studies suggest that the presence of endothelial dysfunction in the coronary circulation is an independent predictor of cardiovascular disease events. The technique is particularly well suited for study of the earliest stages of atherosclerosis in children and young adults, thus providing maximal opportunity for prevention. Numerous studies have demonstrated that brachial artery reactivity improves with risk factor modification and treatment with drugs known to reduce cardiovascular risk. It remains unknown whether an improvement in endothelial function directly translates into improved outcome. In the future, however, practitioners may use brachial artery FMD to assess response to drug therapy and to individualize patient risk factor modification programs. Further studies are needed to determine whether the methodology is sufficiently reproducible and whether biological variability is sufficiently low to make assessment of FMD a clinically useful measure of cardiovascular risk on an individual or group basis[**5**]

Measurement of endothelial function in patients has recently emerged as a useful tool for atherosclerosis research. In the setting of cardiovascular disease (CVD) risk factors, the endothelium loses its normal regulatory functions. Clinical syndromes such as stable and unstable angina, acute myocardial infarction, claudication, and stroke relate, in part, to a loss of endothelial control of vascular tone, thrombosis, and the composition of the vascular wall. Recent studies have shown that the severity of endothelial dysfunction relates to the risk for an initial or recurrent cardiovascular event. Finally, a growing number of interventions known to reduce cardiovascular risk also improve endothelial function. This concept has prompted speculation that endothelial function serves as a "barometer" for cardiovascular health that can be used for patient care and evaluation of new therapeutic strategies

Assessment of conventional risk factor burden is necessary but may not accurately estimate risk of cardiovascular disease[**6**] Most patients in whom myocardial infarction or ischemic stroke develops have one or more conventional risk factors for atherosclerosis, but these risk factors are also prevalent in the general population. As a result, the predictive value of algorithms based on conventional risk factors is unsatisfactory[**7, 8**]. Nearly 40% of adults presenting to cardiology op may be at intermediate risk for a future cardiovascular event when assessed with current algorithms and these individuals may benefit from further risk stratification. The available screening and diagnostic tests have limitations; cardiac stress tests detect only advanced, hemodynamically significant lesions, and conventional coronary angiography is invasive, provides only a "luminogram," and does not identify vulnerable or unstable

plaque. Tests for early detection of atherosclerotic vascular disease are therefore needed to better assess cardiovascular risk in asymptomatic individuals, the main focus of primary prevention.

Noninvasive arterial testing for cardiovascular risk assess ment is based on several important considerations. Alterations in arterial function and structure predate clinical manifestations of occlusive atherosclerotic disease; changes tend to be widespread and are not limited to a single arterial bed. These alterations result from the cumulative effects of known and unknown vascular risk factors that promote formation and progression of atherosclerotic lesions and may also increase the propensity for atherosclerotic plaque rupture . Identification of such abnormalities in accessible peripheral arteries provides a means for early detection of presymptomatic vascular disease and improved cardiovas cular risk stratification.

Arterial ultrasonography and tonometry are attractive modalities for detecting early disease because they are noninvasive and relatively inexpensive. High- resolution ultrasonography can assess arterial dilatation in response to shear stress or pharmacological stimuli (a function that may be compromised early in atherogenesis) and directly examine the arterial wall for early atherosclerotic changes preceding luminal compromise. Arterial tonometry can be used to acquire arterial pulse waveforms to assess arterial stiffness and wave reflection, measures that have been associated with the presence and extent of atherosclerotic vascular disease and cardiovascular events.

OBJECTIVE

Most patients in whom myocardial infarction or ischemic stroke develops have one or more conventional risk factors for atherosclerosis, but these risk factors are also prevalent in the general population. As a result, the predictive value of current algorithms based on conventional risk factors is unsatisfactory.

There is growing evidence that endothelial function serves as a "barometer" for cardiovascular health and assessment of endothelial function will further help in refining risk in an individual patient and therefore guide in intensity of management.With this background, aimed to study

1. To assess the incremental value of non invasive assessment of endothelial function by flow mediated dilatation of brachial artery and aortic pulse wave velocity in risk stratification and management of low and intermediate risk patients
2. To assess the feasibility of inclusion of flow mediated dilatation and aortic pulse wave velocity in routine cardiac clinical practice

MATERIALS AND METHODS

The study was conducted in the Cardiology Department of Madras Medical College ,Government General Hospital ,Chennai-03.

STUDY DESIGN

Patients attending the Cardiology out patient Department for evaluation of chest pain or dyspnoea or referred by Medicine department for evaluation of cardiovascular risk status were initially screened. Those patients in the age group of 30 to 55 years, with no previous cardiovascular disease but with multiple conventional risk factors categorized by Framingam risk score as low or intermediate risk were included and taken up for treadmill ecg stress test. Patients with inconclusive ecg stress test were included in the study. Patients with established cardiovascular diseases like previous myocardial infarction, acute coronary syndromes, stable angina, stroke, peripheral vascular disease, and renal dysfunction, elevated ESR or WBC, active systemic illness, morbid obesity were excluded from the study. All patients gave written, informed consent, and the study was approved by the Human Ethics Committee of Madras Medical College Government General Hospital ,Chennai-03.

A full clinical history and examination was done by a Cardiologist. Baseline demographic data, cardiovascular risk factors, and cardiovascular medications were documented, and a 12-lead electrocardiogram and Echocardiogram was reviewed. Routine biochemical analysis was done. Patients then underwent Flow Mediated Dilatation of Brachial Artery, Aortic pulse wave velocity, Carotid intima medial thickness and then either coronary angiogram or 64 slice CT Angiogram.

CLINICAL EVALUATION

The presence of CAD was defined as a history of MI, coronary revascularization, or typical chest pain with a positive stress ecg. Diabetes mellitus was defined by the use of insulin injections or oral hypoglycemic agents.

Hypertension was defined as an average systolic blood pressure more than140 mm Hg or diastolic blood pressure more than 90 mm Hg on three separate occasions or by the use of antihypertensive medications . Hypercholesterolemia was defined as a fasting total cholesterol level of 200 mg/dl or by the use of a statin. Smoking status was defined as current smoker or reformed/nonsmoker. Significant renal impairment was defined as chronic renal impairment, with a calculated glomerular filtration rate of less than 60 ml/min/1.73 m2 .Ten year coronary heart disease event risk in patients was calculated using the Framingham risk score , using the variables of gender, age, total cholesterol, high-density lipoprotein (HDL) cholesterol, systolic blood pressure, and smoking status. Obesity is defined as BMI >25 kg/m2 and upper limit of normal waist circumference is 80 cm for women and 90 cm for men as defined for asian population.

BIOCHEMICAL ANALYSIS.

Blood for biochemical analysis was obtained from fasting venous samples. Total cholesterol, HDL cholesterol, and triglycerides were determined by standard enzymatic methods. High-density lipoprotein was measured as a homogeneous assay in liquid phase.The Friedewald equation was used to calculate low-density lipoprotein.

FLOW MEDIATED DILATATION.

Endothelium-dependent FMD of the brachial artery was examined noninvasively using an established method of high-resolution vascular ultrasound with an upper arm cuff position. Patients were in fasting state and no intake of caffeinated drinks or smoking 4 hours allowed before the study. All vasoactive medications were stopped 48 hours before study. Brachial-artery two-dimensional and pulsed Doppler flow velocity signals were obtained above the antecubital crease with a 7.5-MHz linear array transducer using a vascular ultrasound system. Hyperemia was induced by inflating a blood pressure cuff on the proximal portion of the arm to occlude arterial flow (^{3}200 mm Hg) for 5 min and then rapidly deflating the cuff. The hyperemic flow is measured by pulsed Doppler within 15 seconds of cuff deflation and the maximum diameter of brachial artery is measured at 60 seconds of deflation

After a 10-min rest period to allow restoration of baseline conditions, nonendothelium-dependent brachial-artery dilation was assessed by obtaining two- dimensional images before and 3 min after administration of sublingual nitroglycerin (NTG) (0.4 mg). An investigator blinded to image sequence and clinical information performed off-line analysis of digitized end-diastolic images .

AORTIC PULSEWAVEVELOCITY.

aPWV can be measured noninvasively, and the technique has

been found to be highly reproducible, with replicate testing yielding an intraclass correlation _0.80. aPWV was measured using commercial available arterial tonometer and pulse wave analysis software system by recording pulse wave signals from the right carotid and right femoral arteries with transcutaneous tonometer probe with ECG synchronization . Digitized data were recorded by custom programming for subsequent analysis. A minimum of 10 beats were averaged for each simultaneous recording site using the QRS for synchronization. Three separate runs were recorded for each participant, and all usable runs were averaged. The distance between the carotid and femoral sampling sites was measured above the surface of the body with a metal tape measure. This was done to avoid overestimation of the distance portion of the aPWV equation. The time differentials between the onset of pulse wave at carotid and femoral (defined as foot of the pressure tracing at each site) sites were divided by the associated distance to produce wave velocity.

CAROTID INTIMA MEDIAL THICKNESS.

The patients also underwent measurement ofcarotid intimal medial thickness by an already described protocol.

CORONARY ANGIOGRAM/64 SLICE CT ANGIOGRAM

The patients were subjected to either invasive coronary angiogram or 64 slice ct angiogram as "gold standard" for documenting coronary artery disease.

STATISTICAL ANALYSIS

All data are expressed as mean value +/-SD or frequency (%), unless otherwise stated. The baseline clinical characteristics of the groups were compared using the two-tailed independent *t* test for continuous variables and the chi-square or Fisher exact test for non-continuous variables, as appropriate. The outcomes were compared using the log-rank test. Independent predictors of events were calculated using Cox proportional hazards regression. The following variables were used first in a univariate model: age, gender, hypertension, hyperlipidemia, total, low-density lipoprotein, and HDL cholesterol, triglycerides, Framingam risk score. diabetes mellitus, current smoking, FMD post cuff and post NTG, aPWV,CIMT. Factors with a value of p less than 0.20 were then entered into a forward stepwise multivariate Cox proportional hazards analysis. Statistical significance was assumed at p less than 0.05. All statistical analyses were performed using SPSS for Windows 11.0 .

RESULTS

CLINICAL CHARACTERISTICS

The study population had 74 patients with 52 men and 22 women. The mean age was 43.96+/- 2.9.Diabetics constituted 35.14% and systemic hypertension was present in 27.4% of study population.There were 17 current smokers [22.97%]. The mean BMI was 26.13, mean waist circumference was 89.15, mean systolic blood pressure was 130.74, mean diastolic blood pressure was 78.93, mean fasting blood sugar was 102.61, mean total cholesterol was 210.20, mean low density lipoprotein was 120.54, mean high density lipoprotein was 45.39, mean triglycerides was 127.89, mean Framingham risk score was 8.76. There were 8 patients with FRS less than 5, 48 patients with FRS between 6 and 10, and 18 patients with FRS more than 10.

IMAGING RESULTS AND OUTCOMES

The mean post cuff FMD was 8.66% +/- 2.64, mean post NTG FMD was 13.98% +/-3.07, mean baseline diameter of brachial artery was 3.68 +/-0.49, mean aortic pulse wave velocity was 11.56 m/s +/-1.64and mean carotid intima medial thickness was 0.65mm +/-0.10.

Out of 74 patients 31 patients had documented coronary artery disease as defined as atleast 50% stenosis in one /more coronary arteries by coronary angiogram/ ct angiogram.

Flow mediated dilatation was initially treated as continuous variable and then as discrete variable.Previous studies have shown that post cuff FMD less than 8% and post NTG FMD less than 15% represent abnormal FMD after taking into consideration the wide biologic variability of FMD.In our study both post cuff [representing nitric oxide mediated endothelium dependent dilatation]and post NTG [represe nting endothelium independent ,direct smooth muscle stimulation] concurred with each other.

Abnormal FMD was present in 30 patients in our study population out of which 21 were men and 9 were women.Among this 30 patients with abnormal FMD 23 patients had documented CAD[17 men and 6 women] . Remaining 7 patients [4 men and 3 women]had no CAD.Among 23 patients with abnormal FMD and documented CAD, carotid intima medial thickness was increased in 14 patients only implying functional changes occur before structural changes atleast in the intermediate risk patient population.Aortic pulse wave velocity was increased in 16 patients in the abnormal FMD plus documented CAD group.Taken together FMD has a positive predictive accuracy of 74.2% whereas carotid intima medial thickness has 51.6% and aortic pulse wave velocity has 58.0% accuracy.

Normal FMD was present in 44 patients[31 men and 13 women] in our study population of 74 patients.Out of this 36 patients[25 men and 11 women] had no CAD and 8 patients[6 men and 2 women] had documented CAD. Among 36 patients [normal FMD plus no CAD group] carotid intima medial thickness was increased in 4 patients and aortic pulse wave velocity was increased in 10 patients. In the 8 patients [normal FMD and documented CAD group] carotid intima medial thickness was increased in 2 patients and aortic pulse wave velocity was increased in 2 patients. Overall this translates into a negative predictive accuracy of 81.2% for FMD whereas carotid intima medial thickness has 72.2% and aortic pulse wave velocity has 68.3% negative predictive accuracy.

When all the three non invasive imaging modalities were combined together the positive predictive accuracy improved only marginally compared to FMD alone, meaning using FMD alone will help in better utilization of resources cutting both cost and time.

CAD and correlation with abnormal FMD did not reveal any significant correlation. Multivariate predictors of CAD in patients with CAD with more than one univariate predictor revealed that in patients with CAD, independent predictors included hyperlipidemia, smoking and abnormal FMD. On comparison with angiographic disease only FMD was statistically significant with a p value less than 0.01 whereas aortic pulse wave velocity with p value of 0.57 and carotid intima medial thickness with a p value of 0.62 were statistically insignificant.

TABLE – 1 CLINICAL CHARACTERISTICS

Variable	Mean	SD
Age	43.96	2.90
BMI	26.13	2.41
Waist Circumference	89.15	7.08
Systolic BP	130.74	11.78
Diastolic BP	78.93	14.81
FBS	102.61	13.05
TC	210.20	36.17
LDL	120.54	15.99
HDL	45.39	4.87
TGL	127.89	23.29
FRS	8.76	2.84

TABLE – 2 CLINICAL CHARACTERISTICS

Variable	No	Percentage
MEN	52	70.27
WOMEN	22	29.73
HTN	20	27.4
DM	26	35.14
SMOKING	17	22.97

TABLE – 3
IMAGING

VARIABLE	MEAN	SD
FMD Post-Cuff	8.66	2.64
FMD Post-NTG	13.98	3.07
Baseline Diameter	3.68	0.49
a PWV	11.56	1.64
CIMT	0.65	0.10

TABLE – 4 APWV

APWV	CAD Group	Normal Group	PPV	NPV	Angiographic Correlation 'P'
Normal APWV (41)	13	28	58.0 %	68.3 %	0.57
Abnormal APWV (33)	18	15			

TABLE – 5 FMD

FMD	CAD Group	Normal Group	PPV	NPV	Angiographic Correlation 'P'
Normal FMD (44)	8	36	74.2 %	81.2 %	< 0.01
Abnormal FMD (30)	23	7			

TABLE – 6 CIMT

CIMT	CAD Group	Normal Group	PPV	NPV	Angiographic Correlation 'P'
Normal CIMT (54)	15	39	51.6 %	72.2 %	0.62
Abnormal CIMT (20)	16	4			

TABLE – 7
FMD Vs Angiographic CAD

	CAD Group		Normal Group	
	Mean	SD	Mean	SD
FMD - Post-Cuff	7.18	2.27	9.73	2.36
FMD - Post-NTG	12.44	2.85	15.09	2.75

DISCUSSION

Many recent studies have shown that endothelial function may serve as an integrating index of risk factor burden and genetic susceptibility, and that endothelial dysfunction will prove to be a valuable independent marker of cardiovascular disease

According to the response-to-injury model of atherosclerosis, various factors can cause dysfunctional alterations in the overlying endothelium. This injury may then predispose arteries to the development of atherosclerosis, eg, by increasing the adhesiveness of the endothelium to leukocytes, by changing its permeability, and by inducing endothelial expression of vasoactive molecules favoring atherogenesis. This model thus predicts that arterial endothelial damage or activation is required before risk factors can induce atherosclerotic changes in the arterial wall

Several risk factors related to atherosclerosis have also been linked to endothelial dysfunction, presumably because of increased oxidative stress. However, recent studies have also shown that individuals with normal endothelial functions and various stages of endothelial dysfunction do not necessarily differ in their risk factor profiles. Al Suwaidi et al observed among 157 patients with mildly diseased coronary arteries that the proportion of hyperlipidemic, hypertensive, or smoking subjects did not differ across the groups with or without endothelial dysfunction. Similarly, Gokce et al found no difference in the proportion of these 3 main risk factors for CHD among 187 patients undergoing vascular surgery between subjects with normal endothelial function and mild or severe dysfunction. **[92, 93]**

In our study we foccused on a narrow but an important spectrum of patient population.Many middle aged men and women presenting with chest pain or dyspnoea and positive for multiple coronary artery disease conventional risk factors after initial clinical , biochemical , resting ecg and echo studies are placed in low or intermediate risk category and subjected to stress ecg. The subjects with positive and negative stress ecg are excluded from the study and managed appropriately. For those subjects with inconclusive stress ecg the next options are dobutamine echo, stress thalium scan, 64 slice ct angiogram or conventional angiogram. We hypothesized that in this cohort of subjects non invasive assessment of endothelial function by brachial artery flow mediated dilatation and aortic pulse wave velocity will further refine the risk and save unnecessary invasive angiograms and will save both cost and time.

In our study population brachial FMD demonstrated a good correlation for the presence of coronary artery disease when conventional angiogram or 64 slice ct angiogram is used as gold standard. Brachial FMD had a positive predictive value of 74.2% and negative predictive value of 81.2% for detection of coronary artery disease. Aortic pulse wave velocity taken alone showed a trend towards a better prediction but was not significant. It had a positive predictive accuracy of 58.0% and negative predictive accuracy of 68.3%. Carotid intima medial thickness a measure of arterial structural alteration to our surprise showed a weak correlation with both brachial FMD and presence of disease.

Both brachial FMD and Aortic pulse wave velocity are continuous variables in a population. Since we were also focusing on the practical utility of both these modalities, after careful and meticulous analysis of previous general population and disease based studies we assigned a cut off value and treated them as diecrete variable.For brachial FMD post cuff value of less than 8% and post NTG value less than 15% represented either lowest tertiles or 75 th percentile in studies. For Aortic pulse wave velocity assigning a discrete value was very difficult and for men a value more than 12.5 m/s and for women a value more than 11.5 m/s is taken as abnormal after referencing the highest and lowest tertiles of population and disease based studies. For carotid intima medial thickness we used well validated age and sex adjusted nomogram.

In our study univariate logistic regression analysis did not show any significant association with individual risk factors and brachial FMD .Systolic blood pressure and age directly correlated with Aortic pulse wave velocity. Age and hyperli pidemia had a direct association with carotid intima medial thickness. Multivariate predictors of CAD in patients with CAD with more than one univariate predictor revealed that in patients with CAD, independent predictors included hyperlipidemia,smoking and abnormal FMD. Significantly there was no correlation between multiplicity of conventional risk factors and Framingham risk model and abnormal FMD in this study population . This fact is in line with concept that FMD could represent a unique measure of vascular health, which may be influenced significantly by parameters currently not measured. Such parameters may overwhelm the influences of traditional risk factors, especially in a population with a low prevalence of traditional risk factors.

Our data did show a positive association between brachial

FMD and aortic pulse wave velocity but it was not significant.The association between brachial FMD and carotid intima medial thickness was also not significant. Together these observations suggest that

1. The endothelial status may not be determined solely by the individual risk factor burden,
2. The status of brachial endothelial function would modify the association between risk factors and atherosclerosis,
3. Brachial flow mediated dilatation represents global endothelial function and has an independent and incremental information over and above the current risk assessment.

CONCLUSION

Brachial artery flow mediated dilatation represents global endothelial function and is significantly associated with the presence of angiographically documented atherosclerosis in a cohort of middle aged south indian population with multiple coronary risk factors.

Aortic pulse wave velocity is weakly associated with both brachial flow mediated dilatation and angiographic disease.

Both brachial flow mediated dilatation and aortic pulse wave velocity correlated weakly with carotid intima media thickness

Thus, brachial flow mediated dilatation when done with meticulous attention to details is safe,inexpensive, rapid, reliable and reproducible and integration of same in routine clinical practice will provide incremental and additive information over conventional risk assessment strategy and better patient care.

Development of guidelines for quality control, standard ization of measurements, and establishment of thresholds for different risk categories will help optimize the use of brachial flow mediated dilatation in clinical practice.