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Research Article

Endothelial dysfunction in patients with metabolic syndrome: a prospective study in a rural institute in India

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ABSTRACT

Background: The objective was to evaluate endothelial dysfunction in patients with metabolic syndrome.
Methods: This prospective, cross-sectional, hospital based observational study included 45 patients with metabolic syndrome and 20 age and sex matched controls who attended hypertension clinic, diabetes clinic, general medicine OPD and patients admitted in wards department of medicine, UPRIMS&R, Saifai, Etawah, UP, India. All patients who fulfilled inclusion criteria were subjected to the color Doppler of the brachial artery in the department of radio-diagnosis of the same institute.
Results: Of the 45 patients with metabolic syndrome included in the study, 22 patients (48.9%) were males and 23 patients (51.1%) were females. Ten males (45.5%) had a waist circumference of >90 cm whereas 20 females (87%) had waist circumference of >80 cm. 9 males (40.9%) had abnormal waist hip ratio whereas 22 females (95.7%) had abnormal waist hip ratio. 43 patients (95.6%) had a SBP >130 mm of Hg whereas 39 patients (86.7%) had a DBP >85 mm of Hg. 24 patients (53.3%) had impaired fasting blood sugar i.e. >100 mg/dl ,25 patients (55.6%) had a TGL level >150 mg/dl, and 9 males (40.0%) had HDL < 40 mg/dl whereas 18 females (78.3%) had HDL value < 50 mg/dl respectively. The mean brachial artery baseline diameter were 3.50 ± 0.39 mm in males and 3.25 ± 0.29 mm in females respectively, FMD percentage was 14.91 ± 3.56 in females and 14.53 ± 4.02 in males, and GTN mediated dilatation were 27.67 ± 9.83% in females and 27.62 ± 6.85% in males respectively.
Conclusion: Estimation of Endothelial Dysfunction in patients at risk of developing full blown Metabolic Syndrome may predict the cardiovascular morbidity and mortality in these individuals even before fulfilling the 3/5 criteria of NCEP/ATP III Guidelines for the diagnosis of metabolic syndrome.

Keywords: Cardiovascular disease, Endothelial function, Flow-mediated vasodilation, Finger plethysmography, Metabolic syndrome, Pulse wave velocity.

INTRODUCTION

The vascular endothelium is a large paracrine organ that secretes numerous factors regulating vascular tone, cell growth, platelet and leukocyte interactions and thrombogenicity. Endothelial dysfunction(ED) can be defined as the partial or complete loss of balance between vasoconstrictors and vasodilators, growth promoting and inhibiting factors, pro-atherogenic and anti-atherogenic factors, and pro-coagulant and anti-coagulant factors.[1]

There are different techniques to evaluate the endothelium functional capacity, that depend on the amount of Nitric Oxide (NO) produced and the vasodilatation effect. The percentage of vasodilatation with respect to the basal value represents the endothelial functional capacity. Taking into account that shear stress is one of the most important stimulants for the synthesis and release of NO, the non-invasive technique most often used is the transient flow-modulate "endothelium-dependent" post-ischemic vasodilatation, performed on conductance arteries such as the brachial, radial or

femoral arteries. This vasodilatation is compared with the vasodilatation produced by drugs that are NO donors, such as nitroglycerin, called "endothelium independent". The vasodilatation is quantified by measuring the arterial diameter with high resolution ultrasonography. Laser-Doppler techniques are now starting to be used that also consider tissue perfusion.

The Metabolic Syndrome represents the co-existence of insulin resistance, hypertension, central adiposity, dyslipidemia, and a pro-inflammatory, pro-thrombotic state. Patients with this syndrome are at increased risk for the development of type 2 diabetes mellitus and cardiovascular disease. Epidemiologic studies reveal a prevalence of the Metabolic Syndrome that increases with age and obesity. Patients with the Metabolic Syndrome should be recognized as being at high risk for cardiovascular complications.[2]

Endothelial dysfunction occurs in various states such as Diabetes, Hypertension, Obesity, Hyperlipidemia, Atherosclerosis, Metabolic Syndrome (Insulin resistant states). Brachial artery flow-mediated dilation (FMD) is a validated, widely used method for gauging endothelial dysfunction due to its noninvasive and practical nature, as pointed out in the study by Calermajer *et al.* [9] Endothelial dysfunction in the brachial artery highly correlates with endothelial dysfunction in the coronary circulation, which is emerging as an independent risk factor for cardiovascular disease. According to Moens et al, FMD is diminished in patients with several coronary risk factors, is an independent predictor of cardiovascular events, and improves with risk reduction therapy.[3]

This study is relevant in the Indian context as there is paucity of data regarding endothelial dysfunction in patients with metabolic syndrome in North Indian population moreso in the rural population. Further, this non-invasive evaluation of endothelial dysfunction by means of color doppler is a cheap, easily available, and reproducible method and it serves as a predictor and marker of future cardiovascular risk and events.

Objective*:* To evaluate endothelial dysfunction in patients with metabolic syndrome.

METHODS

Subjects

This prospective, cross-sectional and hospital based observational study was carried out in the department of internal medicine, Uttar Pradesh Rural Institute of Medical Sciences and Research, Saifai, Etawah, UP, India for a period of one year starting from first of July, 2011.

Forty five patients with Metabolic syndrome and 20 age and sex matched controls were recruited in the study. The subjects attended hypertension clinic, diabetes clinic, general medicine OPD and patients admitted in wards department of medicine, UPRIMS&R, Saifai, Etawah, UP, India. All patients who fulfilled inclusion criteria were subjected to the color Doppler of the brachial artery in the department of radio-diagnosis of the same institute.

Inclusion Criteria

Patients were included on the basis of NCEP/ATP III guidelines for the metabolic syndrome and IDF criteria for central adiposity in Asian Indian phenotypes (three or more of the following):

Clinical identification of metabolic syndrome.

Risk Factor	Defining level
Abdominal obesity	Waist circumference
Males	≥90 cm
Females	≥80 cm
Serum triglycerides	≥ 150 mg/dl
High-density lipoprotein cholesterol	
Males	< 40 mg/dl
Females	< 50 mg/dl
Blood pressure	≥ 130/ ≥ 85 mm Hg or specific medication
Fasting blood glucose	≥ 100 mg/dl or specific medication or previously diagnosed T2 DM.

Exclusion Criteria: Hypoglycemia, Diabetic ketoacidosis, Hyperosmolar non-ketotic coma, Acute myocardial infarction**,** Cerebrovascular accidents, acute infections, Chronic kidney disease, Collagen vascular disorders.

Study Design: This is a prospective, cross-sectional study.

An informed consent was obtained from each subject prior to entering the study. Clinical examination included blood pressure measurement, cardiovascular examination, ECG, anthropometrical measurements, and body-mass index (BMI). Biochemical assessment included fasting blood sugar (FBS) and post-prandial blood sugar levels, Renal function tests (Blood urea & serum creatinine), urine for microalbumin, and Serum lipid profile. Plasma glucose and lipid estimation were done after an overnight fast of 12 hour.

Assessment of Endothelial Dysfunction by Color Doppler of Brachial Artery

The brachial artery was imaged above the antecubital fossa in the longitudinal plane. A segment with clear anterior and posterior intimal interfaces between the lumen and vessel wall was selected for continuous 2D grayscale imaging.

Endothelium-dependent FMD: To create a flow stimulus in the brachial artery, a sphygmomanometric (blood pressure) cuff was first placed above the antecubital fossa. A baseline rest image was acquired, and blood flow was estimated by time-averaging the pulsed Doppler velocity signal obtained from a mid-artery sample volume. Thereafter, arterial occlusion was created by cuff inflation

to supra systolic pressure. Typically, the cuff was inflated to at least 50 mmHg above systolic pressure to occlude arterial inflow for a standardized length of time. This causes ischemia and consequent dilation of downstream resistance vessels via autoregulatory mechanisms. Subsequent cuff deflation induced a brief high-flow state through the brachial artery (reactive hyperemia) to accommodate the dilated resistance vessels. The resulting increase in shear stress caused the brachial artery to dilate. The longitudinal image of the artery was recorded continuously from 30 seconds to 2 min after cuff deflation. A mid-artery pulsed Doppler signal was obtained upon immediate cuff release and no later than 15s after cuff deflation to assess hyperemic velocity.

Endothelium-independent vasodilatation with nitroglycerin: At least 10 min of rest was needed after reactive hyperemia (FMD) before another image was acquired to reflect the re-established baseline conditions. An exogenous NO donor, such as a single high dose (0.4 mg) of nitroglycerin(NTG) spray or sublingual tablet was given to determine the maximum obtainable vasodilator response, and served as a measure of endothelium-independent vasodilatation reflecting vascular smooth muscle function. Peak vasodilatation occurred 3 to 4 min after NTG administration; images should be continuously recorded during this time.

Analysis

Characterizing FMD: Flow-mediated vasodilatation is typically expressed as the change in post-stimulus diameter as a percentage of the baseline diameter. Similarly endothelium independent vasodilatation is calculated. The difference between the two percentages is taken as a marker for Endothelial Dysfunction.[4] Statistical analysis was performed using SPSS version 14.0 Statistical package for windows (SPSS, Chicago, IL). Paired and students't' test, Multivariate regression analysis was done. $p < 0.05$ was taken as statistically significant.

RESULTS

In the 45 patients studied 22 (48.9%) were males and 23(51.1%) were females with an age range of 34 to 70 years. Table 1 denotes the demographic profile of patients with metabolic syndrome.

Ten males (45.5%) had a waist circumference of >90 cm whereas 20 females (87%) had waist circumference of >80 cm. 9 males (40.9%) had abnormal waist hip ratio whereas 22 females (95.7%) had abnormal waist hip ratio.43 patients (95.6%) had a SBP >130 mm of Hg whereas 39 patients (86.7%) had a DBP >85 mm of Hg. 24 patients (53.3%) had impaired fasting blood sugar i.e. >100 mg/dl, 25 patients (55.6%) had a TGL level >150 mg/dl, and 9 males (40.0%) had HDL < 40 mg/dl whereas 18 females (78.3%) had HDL value < 50 mg/dl respectively.

Table 1: Demographic Profile.

	Mean	Standard Deviation
Age (years)	54.36	10.71
Weight (kg)	72.29	10.6
Height(cm)	160.33	7.39
BMI (kg/m$^{2)}$	28.81	5.16
Waist (cm)	100.56	9.06
Hip (cm)	100.82	8.22
Waist hip ratio	0.997	0.035
SBP(mm Hg)	155.82	15.44
DBP(mm Hg)	94.18	9.4
BS (F) (mg%)	118.56	29.57
BU (mg%)	39.91	5.95
SCR (mg %)	0.86	0.28
Urine for Microalbumin	0.5	0.63
TCHOL (mg %)	183.6	29.65
TGL (mg%)	152.69	27.57
HDL (mg %)	41.84	6.24
LDL (mg %)	85.91	16.12
VLDL (mg%)	39.49	8.89
Endothelial dysfunction (%)	12.92	8.34

Assessment of endothelial dysfunction by means of colour Doppler of brachial artery

The mean brachial artery baseline diameter were 3.50 ± 0.39 mm in males and 3.25 ± 0.29 mm in females respectively, FMD percentage was 14.91 ± 3.56 in females and 14.53 ± 4.02 in males, and GTN mediated dilatation were 27.67 ± 9.83% in females and 27.62 ± 6.85% in males respectively. These results indicate that FMD is depressed implying thereof endothelial dysfunction in the study group. (GTN-Glyceryl Tri Nitate).

Table 2: Brachial artery measures.

	Baseline diameter (mm)	Flow mediated dilatation (FMD)		GTN mediated dilation		Endothelial dysfunction
		(mm)	(%)	(mm)	(%)	(%)
Female	3.25±0.29	3.73±0.33	14.91±3.56	4.29±0.35	27.67±9.83	12.76±9.83
Male	3.50±0.39	4.00±0.36	14.53±4.02	4.58±0.34	27.62±6.85	13.09±6.67

Values given as mean ± SD

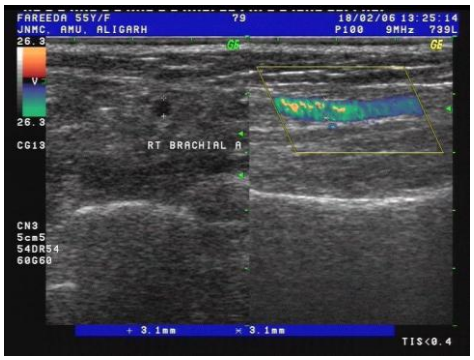

Figure 1: Baseline Diameter of Right Brachial Artery.

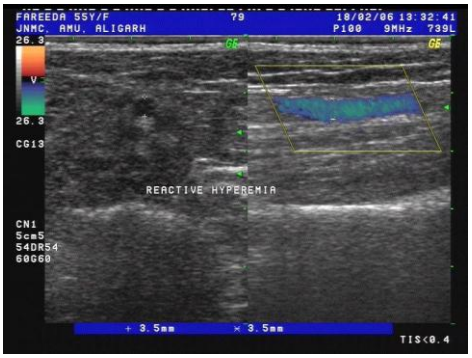

Figure 2: Diameter of Brachial Artery After Reactive Hyperemia.

(SBP-Systolic Blood Pressure, DBP-Diastolic Blood Pressure, BS(F)-Blood Sugar Fasting, TGL-Triglyceride, HDL-High Density Lipoprotein, BMI-Body Mass Index, ED-Endothelial Dysfunction, BU-Blood Urea, LDL-Low Density Lipoprotein, NS-Non Significant.)

Table 3: Carl Pearson's Product Moment Correlation (r) of Endothelial dysfunction with other variables.

	'r'	P
SBP	0.338	0.023
DBP	-0.001	NS
BS(F)	0.488	0.002
TGL	0.488	0.001
HDL	-0.656	< 0.001
BMI	0.436	0.003
Waist hip ratio	-0.121	NS
BU	0.385	0.009
LDL	0.477	0.001
Age	0.506	< 0.001

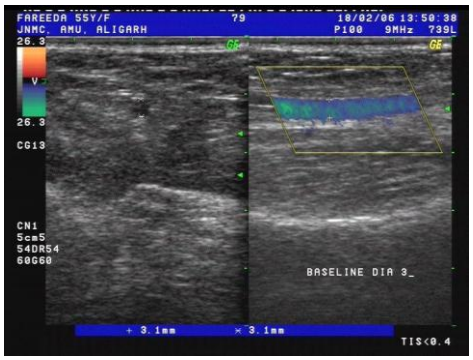

Figure 3: Baseline Diameter of Right Brachial Artery 10 minutes after reactive hyperemia.

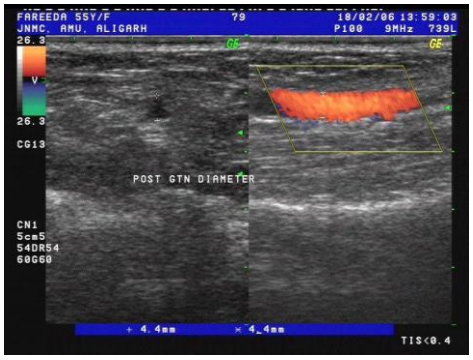

Figure 4: Post GTN (Glyceryl Tri Nitrate) Diameter of Right Brachial Artery.

Univariate analysis of different variables with Endothelial Dysfunction was done using Carl Pearson's Correlation. (Table 3) SBP, BS (F), TGL, HDL, BMI were significantly correlated with ED while DBP and Waist hip ratio were not. Further on comparing the Endothelial dysfunction between cases and controls using T- test, a significant higher percentage was found in the cases as compared to controls.

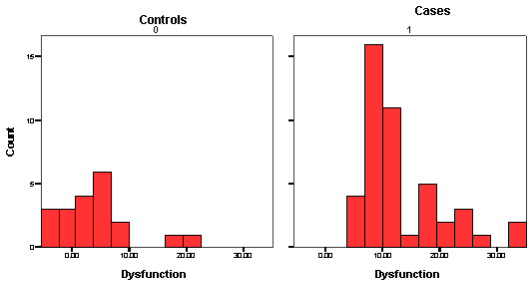

Figure 5: Distribution of Control and Case Frequencies in relation to Endothelial Dysfunction.

Figure 5 summarizes that there is significant difference between cases and controls and cases are high on the counts of endothelial dysfunction

Stepwise multiple regression analysis

The table (4) illustrates a stepwise multiple regression model with Endothelial Dysfunction as the dependant variable and HDL, fasting blood glucose, SBP, Age and TGL as independent variables. The step wise multivariable predictors of endothelial dysfunction in the present study were high density lipoprotein (HDL), blood sugar fasting (BSF), systolic blood pressure (SBP), age and low density lipoprotein (LDL). HDL was the most important predictor of endothelial dysfunction with 66.4% predictability, 44.1% variance (R square) and β = -0.254. Only HDL was inversely correlated with endothelial dysfunction where as all other variables were directly associated with endothelial dysfunction (ED).

Table 4: Model summary & coefficient for endothelial dysfunction.

	Variables	R	R Square	β	95% Confidence Interval	
					Lower	Upper
1	HDL	0.664	0.441	-0.254	-0.029	-0.479
2	BS (F)	0.770	0.593	0.092	0.049	0.135
3	SBP	0.813	0.661	0.175	0.078	0.272
4	Age	0.838	0.703	0.201	0.051	0.352
5	LDL	0.860	0.740	0.105	0.012	0.198

DISCUSSION

Endothelial dysfunction is a harbinger of many cardiovascular diseases The assessment of endothelial dysfunction is a rational approach for risk assessment of patients with or at risk for cardiovascular disease.[5] Brachial artery flow-mediated dilation (FMD) is a surrogate marker of endothelial vasodilator function in humans.[6] Studies demonstrate that endothelial dysfunction precedes the development of clinically apparent atherosclerosis in individuals with CVD risk factors as smoking, hypertension, hyperlipidemia diabetes mellitus, and obesity.[7,8,9] Treating of risk factors may reverse endothelial dysfunction.[10] Finally, endothelial dysfunction is a precursor of future CVD events.[11]

Endothelial dysfunction and Dyslipidemia: Asian Indians have hypertriglyceridemia, low HDL-C, and high levels of small-dense low-density lipoprotein (LDL), it characterises insulin resistance and is labelled as 'atherogenic dyslipidemia'.[12]

CAD patients have impaired Endothelial dependent and independent functions, FMD correlates with serum HDL-C levels on univariate and multivariate analysis.[13] Increased high-density lipoprotein concentration in patients of hypercholesterolemia improves endothelial function.[14] In our study, serum HDL (a risk factor for CAD) inversely correlated ($r = -0.656$, $p < 0.001$) with ED on multivariate analysis, in unison with prior listed studies. In addition, serum cholesterol and LDL, have been inversely associated with FMD in prior studies, also consistent with the present study.[15]

Endothelial dysfunction and Glucose tolerance: Endothelial dysfunction preceeds the vascular manifestations of Diabetes Mellitus. DM leads to endothelial dysfunction by multiple pathways, mostly as a result of insulin resistance.[16]

Obesity, insulin resistance, and endothelial dysfunction are closely linked in the natural history of type 2 diabetes. Visceral fat-derived metabolic products, hormones, and cytokines play a major role in affecting insulin action in skeletal muscle resulting in low-grade inflammation and endothelial dysfunction. Some shared insulin signaling abnormalities in muscle, fat, and endothelial cells may play role in this causation.[17]

In our study, ED correlated directly with impaired fasting glucose ($r = 0.488$, $p < 0.05$) and LDL levels ($r = 0.477$, $p < 0.001$), similar to the study conducted by Frangi *et al*.[18]

On stepwise multivariate analysis, age, fasting plasma glucose were important contributing factors for ED in accordance with the study conducted by Sonka *et al.*[19]

Endothelial dysfunction and Hypertension: Our study provides evidence that SBP is an important correlate of endothelial dysfunction. Indeed, the gradient of diminished FMD begins at non-hypertensive BP levels. A causal relationship, between hypertension and endothelial dysfunction has been observed in earlier studies.[20] Also non-obese individuals have endothelial dysfunction, suggesting that hypertension causes endothelial dysfunction independent of the effect of weight.[21]

Antihypertensive treatment improves-endothelial function and these patients have fewer future CVD events.[22,23] The causal relationship between endothelial dysfunction and hypertension is not clear. Studies show that endothelial dysfunction may cause essential hypertension.[24] However, the present cross-sectional study cannot determine whether endothelial dysfunction, is a cause or consequence of hypertension, or alternatively, whether FMD and SBP were associated by virtue of their joint correlation with a third factor, such as arterial stiffness.[25]

Endothelial dysfunction and age: Endothelial function decreases with advancing age, but the mechanisms is unclear and is possibly due to increase in reactive oxygen species production with increasing age.[26,27,28] And in our study, age is significantly correlated to Endothelial Dysfunction.

Endothelial dysfunction and Obesity: Asian Indians have higher proportion of body fat and lower body mass index (BMI), high waist-hip ratio (WHR) with low waist circumference and are leaner and thinner against Causacians counterparts.[29] A direct association between BMI and Endothelial Dysfunction was found in our study consistent with earlier studies.[30,31] Endothelial Dysfunction improved with weight loss.[32] The possible causal factors may be oxidative stress and systemic inflammation.[33] However, we did not find that BMI was a multivariable predictor of ED. A plausible explanation may be that increased body fat and decreased lean body mass may compensate each other not allowing appreciable increase in the value of BMI. This may aptly explain that BMI does not reflect adiposity accurately in Asian Indians.

The most striking finding in our study was the predictability of our covariates on the outcome of Endothelial Dysfunction in Stepwise Multiple Regression Model. HDL was the most significant of all the predictors accounting for 66.4% predictability, 44.1% of the total variance (R square) and β= -0.254. Only HDL was inversely associated with ED whereas all other variables were directly related to ED. However a larger and further prospective studies are required to strengthen our study findings so that in the future it may be applicable and useful to the masses.

Whereas BMI, blood urea and TGL were associated with impaired ED in univariate analysis, they were not retained in the multivariate regression model. There are several plausible explanations for the apparent dissimilarity with prior research. A reasonable explanation may be that increased body fat and decreased lean body mass may compensate each other and appreciable increase in the value of BMI is negated. The lack of independence of the variables could be attributed to the clustering of hypertension, glucose intolerance, lipid abnormalities, and obesity in individuals with the metabolic syndrome, resulting in colinearity among these variables.[34] Our study may have potential confounders because of the small sample size and community-based design. Alternatively, it may have been difficult to detect these relations because we studied middle-aged-to-elderly individuals with a typical distribution of lipid and glucose values and relatively low mean FMD% and more ED. Studies show that in the presence of multiple competing risk factors, the combined effect may overwhelm the particular effect of the risk factor.[35]

So large prospective studies are needed to assess the strength of association of brachial artery FMD or other ED markers with Metabolic syndrome and future cardiovascular events. The present study shows that ED is associated with components of Metabolic Syndrome which is also an independent risk factor for CAD/CVD. Assessment of ED should be included along with other conventional risk variables such as lipoproteins to identify individuals at increased risk for CAD and extended Cardio-metabolic syndrome at an early stage, given the paucity of facilities for invasive evaluation in India.

CONCLUSION

- Estimation of ED in patients at risk of developing full blown Metabolic Syndrome may predict the cardiovascular morbidity and mortality in these individuals even before fulfilling the 3/5 criteria of NCEP/ATP III Guidelines for the diagnosis of Metabolic Syndrome.
- We therefore recommend that evaluation of Endothelial Dysfunction by brachial artery flow mediated dilatation (FMD) should be included in the diagnostic criteria of the expanded Cardio-Metabolic Syndrome as other markers like microalbuminuria, apo-lipoprotein β, Tumour Necrosis Factor (TNF-α), Interlukein-1 and 6 (IL-1, IL-6).
- Endothelial Dysfunction is a surrogate marker and predictor of pre-Metabolic Syndrome.

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Ethical approval: The study was approved by the local ethics committee

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