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A STUDY ON ENDOTHELIAL DYSFUNCTION IN DIABETES MELLITUS IN SIDDHARTHA GOVERNMENT GENERAL HOSPITAL VIJAYAWADA

KEYWORDS: Endothelial dysfunction, Diabetes mellitus, Flow mediated dilatation(FMD).

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ABSTRACT Diabetes is assuming epidemic proportions globally and India would emerge as the diabetic capital of the world in the foreseeable future. The loss of the modulatory role of endothelium may be the initializing and critical factor in diabetic vascular disease. Endothelial dysfunction is believed to be the earliest functional abnormality in diabetes and may serve as a very important surrogate marker for future atherosclerosis among the diabetic population.In view of the facts that diabetes and its macro and micro vascular complications are very common in our country, the role of endothelial dysfunction in causing the above complications. The present study was undertaken with the following aims at studying endothelial dysfunction in patients of type 2 Diabetes mellitus and to compare it with age and sex matched control subjects, and to compare endothelial dysfunction among patients of type 2 diabetes mellitus and controls.

INTRODUCTION

Diabetes is assuming epidemic proportions globally and it is projected that by the year 2025, there would be 300 million diabetics worldwide and India would emerge as the diabetic capital of the world. The worldwide prevalence of DM has risen dramatically over the past two decades, from an estimated 30 million cases in 1985 to 382 million in 2013. Based on current trends, the International Diabetes Federation projects that 592 million individuals will have diabetes by the year 2035. The metabolic dysregulation associated with DM causes secondary patho-physiologic changes in multiple organ systems with both micro vascular and macro vascular complications. Globally, DM is the leading cause of end-stage renal disease (ESRD), non traumatic lower extremity amputations, and adult blindness. It also predisposes to cardiovascular diseases. This may be accompanied by other biochemical disturbances and the presence of progressive tissue damage. The mortality and morbidity in diabetes are mostly attributed to the micro and macro vascular complications of the disease. The loss of modulatory role of endothelium may be the initializing and critical factor is diabetic vascular disease. Endothelial dysfunction is believed to be the earliest functional abnormality in diabetes and serves as a very important surrogate marker for future atherosclerosis among them.[1] Injury to the endothelium causes endothelial dysfunction. Failure of the endothelium to elicit nitric oxide (NO) mediated vasodilatation may be due to decreased formation, increased degradation, decreased sensitivity to NO formed or a combination of these factors. Irrespective of the mechanisms involved this is referred to as endothelial dysfunction. Endothelial dysfunction as assessed in terms of vasomotor dysfunction can occur well before the structural manifestations of atherosclerosis. Assessment of endothelial function, thus, can provide valuable insight into the pre- intrusive phase of atherosclerosis and can be used as an early marker of future atherosclerotic disease. However, the invasive nature of the earlier available tests for assessment of endothelial function precluded their use in clinical practice.

Development of non-invasive method of assessment of endothelial function by brachial artery flow mediated vasodilatation (FMD) provided an extremely useful tool for cardiovascular research and for clinical application.[2] This test can be done easily and has proven reproducibility.[3]

In view of the above facts,[4,5,6] the present study has been undertaken to determine occurrence of endothelial dysfunction in Type 2 DM and to compare the dysfunction with that of non diabetic population.

MATERIAL AND METHODS

50 Diabetic patients, attending the OPD or admitted in the wards of, The Department of General Medicine, SIDDHARTHA MEDICAL COLLEGE, VIJAYAWADA during the period from December 2014 to November 2016 were included in the study. Informed consent was obtained from all the study subjects.50 age and sex matched healthy subjects served as controls. Informed consent was obtained from subjects belonging to the control group. Thus the subjects in the present study were divided into two groups, patients with diabetes (n=50) and patients without diabetes (n=50).

INCLUSION CRITERIA

1. Age 30- 75yrs

2. Both sexes.

3.Newly detected type- 2 Diabetes Mellitus or type -2 Diabetes ellitus on treatment with OHA or insulin or both.

Diabetes Mellitus was diagnosed as per ADA (2011) guidelines.

EXCLUSION CRITERIA

Age < 30 yrs and > 75yrs, type – 1 Diabetes mellitus, co-morbid illness which are likely to influence endothelial function, hypertension, congestive cardiac failure, liver disease, chronic infections and Patients who did not give consent for the study.

Subject preparation

Numerous factors affect flow-mediated vascular reactivity, including temperature, food, drugs and sympathetic stimuli, among others. Therefore, subjects should fast for at least 8 to 12 h before the study, and they should be studied in a quiet, temperature-controlled room. All vasoactive medications should be withheld for at least four half-lives, if possible. In addition, subjects should not exercise, should not ingest substances that might affect FMD such as caffeine, high-fat foods and vitamin C or use tobacco for at least 4 to 6 h before the study.

Clinical examination included blood pressure measurement, cardiovascular examination and body mass index (BMI). Biochemical assessment included fasting blood sugar (FBS) and postprandial blood sugar levels, and comprehensive lipid profile. Plasma glucose & lipid estimation were done after an overnight fast for at least 8hrs. Assessment of flow mediated dilatation in Brachial artery by Colour

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Doppler Ultra-sonography was performed in all subjects using 7.5 MHz probe attached to ESOATE MYLAB CLASS C HIGH END ultrasound machine. The brachial artery diameter was measured on B- mode ultrasound images. The Right brachial artery was studied in all the subjects.

Scans were obtained with the subject at rest, during reactive hyperaemia, and again at rest. The subjects were asked to lie quietly for at least 10 min before the first scan. The brachial artery was scanned in longitudinal section 2 - 15cm above the elbow, and the centre of the artery was identified when the clearest picture of the anterior and posterior intimal layers was obtained. The transmit (focus) zone was set to the depth of the near wall, because of the greater difficulty in evaluating the "m" line

(the interface between the media and adventitia) of the near wall as compared with that of the far wall. Depth and gain settings were set to optimize images of the interface between the lumen and the arterial wall, and the images were magnified. Settings for operating the machine were not changed during the study.

When a satisfactory transducer position was found, the skin was marked and the arm was kept in the same position throughout the study. A resting scan was obtained, and the velocity of arterial flow measured with a pulsed Doppler signal at a 70o angle to the vessel, with the range gate (1.5mm) in the centre of the artery.Increased flow was then induced by the inflation of a sphygmomanometer cuff placed around the right arm to a pressure of 50mm Hg above systolic blood pressure for 5 min, followed by release. After release of the cuff the brachial artery diameter was measured again at 1 minute to assess FMD.

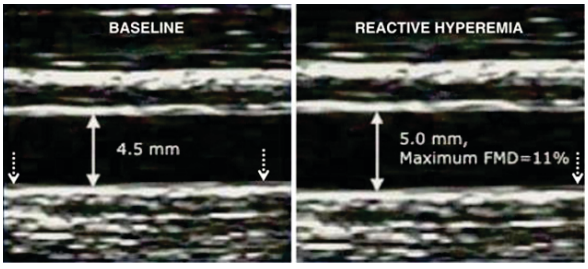


Figure 1: Reactive Hyperemia in response to ischemia
Flow mediated dilatation was calculated, and the average results of the two observations recorded. Flow mediated dilatation was presented as the percent change from base line to hyperemia. Severe endothelial dysfunction was defined as FMD < 4.5% as has been described.

Significant figures
+ Suggestive significance (P value: 0.05<P<0.10)
*Moderately significant (P value:0.01<P ≤ 0.05)

**Strongly significant (P value : P≤0.01)

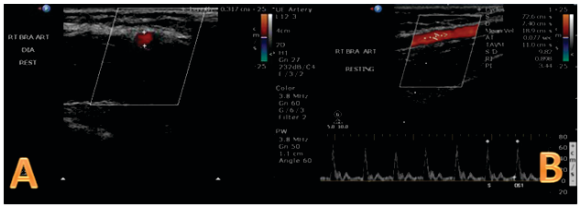


Figure A: Right Brachial Artery Colour Doppler showing Baseline Diameter before compression of 3.17 mm. Figure B: Right Brachial Artery Colour Doppler showing Mean Velocities during systole and diastole before compression.

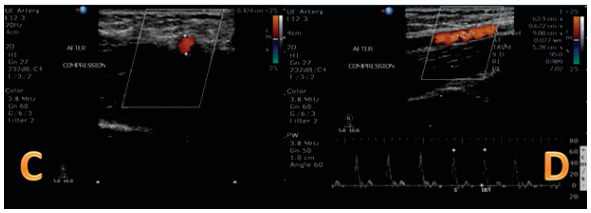
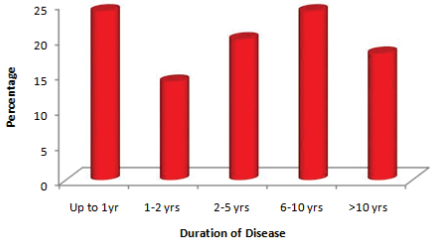


Figure C: Right Brachial Artery Colour Doppler showing Baseline Diameter after compression of 4.24 mm. **Figure D:** Right Brachial Artery Colour Doppler showing Mean Velocities during systole and diastole after compression.

OBSERVATIONS AND RESULTS
Table 1: Age distribution of patients studied

Age in years	Cases		Controls	
	No	%	No	%
<30	1	2.0	1	2.0
30-40	5	10.0	3	6.0
41-50	10	20.0	10	20.0
51-60	12	24.0	19	38.0
61-70	17	34.0	17	34.0
>70	5	10.0	0	0.0
Total	50	100.0	50	100.0
Mean ± SD	58.98±13.06		56.32±9.96	

Samples are age matched with P=0.255. Samples are gender matched with P=1.000
Graph 1:



Mean ± SD: 6.66±6.45
Table 2: Treatment modalities

	No. of patients (n=50)	%
OHA	26	52.0
Insulin	14	28.0
Both	10	20.0

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Graph 2: Distribution of BMI(Body Mass Index) and WHR (Waist-Hip Ratio) in the two the groups studied

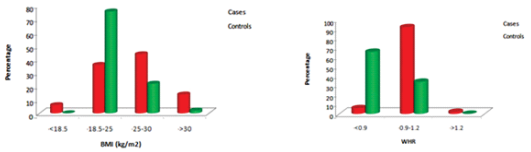

Table 3: Distribution of BD, HF% and FMD% in the two groups studied.

		Cases		Controls		
variables	**(n=50)**		**(n=50)**		**P value**	
		No	**%**	**No**	**%**	
Baseline Diameter(BD) mm						
	<3	**2**	**4.0**	**10**	**20.0**	
						0.021*
	3-4.5	**46**	**92.0**	**40**	**80.0**	
	>4.5	**2**	**4.0**	**0**	**0.0**	
Hyperemic Flow (HF)%						
	<50	**21**	**42.0**	**3**	**6.0**	
						<0.001 **
	50-100	**20**	**40.0**	**20**	**40.0**	
	>100	**9**	**18.0**	**27**	**54.0**	
Flow Mediated Dilatation (FMD)%						
	<4.5	**25**	**50.0**	**0**	**0.0**	
	4.5-15	**9**	**18.0**	**24**	**48.0**	**<0.001 ****
	15-30	**15**	**30.0**	**18**	**36.0**	
	>30	**1**	**2.0**	**8**	**18.0**	

DISCUSSION AND ANALYSIS

FMD is a non invasive investigation to diagnose the presence of endothelial dysfunction in the diabetic population. We have studied FMD in 50 cases and compared the results with FMD done in another 50 control or non diabetic population.

Chi-square/ Fisher Exact test has been used to find the significance of study parameters on categorical scale between two or more groups, non-parametric setting for Qualitative data analysis. The following observations were noted.

In the present study the predominant age of distribution of cases was between61 to 70 years (Mean age = 58.98±13.06), where as the predominant age of distribution of the control population was between 51 to 60 years (Mean age = 56.32±9.96) Males constituted 54 % of both cases and control groups (27 in each group). Females constituted 46 % of both cases and control groups (23 in each group). Among the diabetic population the duration of the disease varied with most people having been diagnosed with diabetes and taking diabetic medication, either between 6 to 10 years (24 % of the cases) or recently diagnosed i.e within 1 year (24 % of the cases). In 20 % of the cases diabetes was diagnosed and were on treatment for a period of 2 to 5 years. 18 % of the cases have been diagnosed as diabetic and have been using medication for a period greater than 10 years.

We have also observed that around 52 % of the cases (26 cases) were only on oral hypoglycemics, where as 28 % of the cases (14 cases) were using only insulin. This indicates that a vast majority of type 2 diabetics use oral medication in preference to insulin. 20 % of the cases (10 cases) use both oral drugs in combination with insulin.

On examination of the anthropometric data the average BMI of the diabetic population was 25.55±4.28 kg/m2 which was in the overweight range (i.e 25 to 29.99 kg/m2). Whereas the BMI of the non diabetic population was 23.21±3.43 kg/m2 which was within the normal range (normal BMI is 18.5 to24.99 kg/m2). Also there was significant difference in the waist to hip ratio. WHR in the non diabetic population was 0.87±0.06 as compared to the diabetic population which was 1.01±0.11 ,which is considerably higher. This indicates that a higher BMI and WHR are risk factors for Endothelial Dysfunction. Majority of the cases , i.e 22 diabetics (44 % of cases) have BMI between 25 to 29.99 whereas 38 non diabetics (76 % of non diabetics) have BMI between 18.5 to 24.99. WHR of < 0.9 is present in 6 % of the cases ; WHR between 0.9 to 1.2 is seen in 92 percent of the cases; WHR > 1.2 is seen in only 2 % of the cases. Both fasting and postprandial blood sugars were analyzed in the study.

Among diabetics FBS levels of greater than 126 mg/dl was noted in 36 cases (72 % of the diabetics). Only 7 cases (14 % of the diabetics) had a glycemic control with euglycemia with FBS less than 100mg/dl. Elevated PPBS levels were seen in 37 cases (74 % of the diabetics) with PPBS greater than 200 mg/dl. 13 cases (26 % of the diabetics) had PPBS levels between 140 and 200 mg/dl (i.e impaired glucose tolerance). Surprisingly none of the diabetics had PPBS levels less than 140 mg/dl indicating poor glycemic control. The BD, i.e baseline diameter of the brachial artery as measured by the Ultrasound Doppler is approximately equal in both the diabetics (3.74±0.47 mm) and non diabetics (3.44±0.51mm) indicating baseline diameter cannot be used as a marker for endothelial dysfunction. Majority of both cases and controls have baseline diameter between 3 and 4.5 mm.

HF% ,which is the measure of the maximum blood flow immediately after the release of the sphygmomanometer cuff is significantly decreased in the diabetic individuals (69.79±60.15%) as compared to the non diabetic individuals (125.52±50.78 %) indicating impaired relaxation of the vessel wall immediately, post hypoxemia .

In the present study there was an impairment of flow-mediated dilatation among diabetics when compared with normal subjects (10.32±9.98% vs 17.38±9.92%), p value =0.001. This indicates an impaired relaxation of the vessel wall due to the decreased release of NO from the endothelium due to its dysfunction.

Table 4: Incidence of ED in two groups studied

ED	Cases		Controls	
	No	**%**	**No**	**%**
Absent	**25**	**50.0**	**50**	**100.0**
Present	**25**	**50.0**	**0**	**0.0**
Total	**50**	**100.0**	**50**	**100.0**

P<0.001**, Significant, Chi-Square test

This indicates almost 50 percent of diabetics are suffering from severe endothelial dysfunction and hence are at increased risk of coronary and cerebral arterial diseases and atherosclerosis in the future.

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Similar results were obtained in other studies. In a study by K. Bhargava et al[6] significant endothelial dysfunction was observed in Diabetics (5.51±2.21%) when compared with normal subjects (7.03±2.87%)6. They have shown that a similar degree of endothelial dysfunction occurred in patients with CAD without diabetes mellitus (4.56±2.7%). In a similar study conducted by Uday Jadhav et al[7] significant impairment of endothelial function occurred in diabetics when compared with non diabetics (p value <0.05)[7]. They have also shown that the endothelial dysfunction was more in diabetic and dyslipidemic population . However, their values were not statistically significant.

Clarkson et al, in their study have shown that endothelial function was significantly impaired in diabetics as compared to controls (5.0±3.7 vs 9.3±3.8%, p value <0.001)[8]. Similarly Yu HI et al, have demonstrated that FMD of the brachial artery was significantly impaired in diabetes. The impairment was more marked in the presence of peripheral arterial disease, dyslipidemia and diabetic complications[9]. Yerong Yu et al have shown that compared with type 2 diabetes patients with normoalbuminuria, patients with micro albuminuria had more pronounced endothelial dysfunction (9.7±4.3 vs 8.0±3.8%, p value <0.05) [10].

Antonaides et al [11]have shown that endothelial function was better in healthy controls when compared with patients with diabetes and CAD (p value <0.001) or patients with diabetes alone (p value <0.001)[11].
In the present study, the incidence of Severe Endothelial Dysfunction , i.e ,

FMD < 4.5 % is seen in 50 % of the diabetics (25 patients with diabetes have severe endothelial dysfunction). FMD of 4.5 – 15 % is seen in 18 % of the diabetics (9 patients with diabetes). FMD of 15 – 30 % is seen in 30 % of the diabetics (15 patients with diabetes) whereas the incidence of FMD > 30 % is seen in only 2 % of the diabetics (only 1 patient with diabetes). In non diabetics no single individual has been diagnosed to have FMD less than 4.5 %. FMD of 4.5 – 15 % is seen in 48 % of the non diabetics (24 individuals). FMD of 15 – 30 % is seen in 36 % of the non diabetics (18 individuals) whereas the incidence of FMD > 30 % is seen in only 18 % of the non diabetics (9 individuals).

Hence the overall incidence of Endothelial Dysfunction in Diabetic population, in our study is 50 % (i.e 25 individuals), whereas no endothelial dysfunction was found in the non diabetic population.

MEASUREMENT OF BRACHIAL ARTERY FMD

The FMD was determined with the occlusion cuff on the upper arm the base line , occlusion and responsive data while performing FMD on brachial artery were assessed and the data is populated on the graph between normal individuals and the people having diabetes. The brachial artery diameters were measured. In diabetic individuals the following curve was not observed. Instead there was both a decrease in the peak flow reached as indicated by HF % immediately after the cuff release and also there was a decrease in the blood flow 1 minute after the cuff release, indicated by FMD % as compared to non diabetic individuals.

ENDOTHELIUM-DEPENDENT FMD

To create a flow stimulus in the brachial artery, a sphygmomanometric (blood pressure) cuff is first placed either above the ante-cubital fossa or on the forearm. A baseline rest image is acquired, and blood flow is estimated by time-averaging the pulsed Doppler velocity signal obtained from a mid-artery sample volume.

Studies have variably used either upper arm or forearm cuff occlusion, and there is no consensus as to which technique provides more accurate or precise information. When the cuff is placed on the upper part of the arm, reactive hyperaemia typically elicits a greater percent change in diameter compared with that produced by the placement of the cuff on the forearm. This may be due to a greater flow stimulus resulting from recruitment of more resistance vessels or possibly due to direct effects of ischemia on the brachial artery. However, upper-arm occlusion is technically more challenging for accurate data acquisition as the image is distorted by collapse of the brachial artery and shift in soft tissue.

The change in brachial artery diameter after cuff release increases as the duration of cuff inflation increases from 30 s to 5 min. The change in diameter is similar after 5 and 10 min of occlusion; therefore, the more easily tolerated 5-min occlusion is typically used. This phenomenon is due to the endothelium derived NO, and hence the process is known as endothelium dependent vasodilatation.

ENDOTHELIUM-INDEPENDENT VASODILATATION WITH NITROGLYCERIN

At least 10 min of rest is needed after reactive hyperaemia (i.e., FMD) before another image is acquired to reflect the re established baseline conditions. In most studies, an exogenous NO donor, such as a single high dose (0.4 mg) of nitroglycerin (NTG) spray or sublingual tablet has been given to determine the maximum obtainable vasodilator response, and to serve as a measure of endothelium-independent vasodilatation reflecting vascular smooth muscle function. Peak vasodilatation occurs 3 to 4 min after NTG administration; images should be continuously recorded during this time, and NTG should not be administered to individuals with clinically significant bradycardia or hypotension. Determining the vasodilator responses to increasing doses of NTG, rather than a single dose, may further elucidate changes in smooth muscle function or arterial compliance that might be playing a role in any observed changes in FMD. Hence it is of absolute essentiality to measure even the vasodilatation after injection of NTG. Absence of dilatation in response to both internal hypoxia and external NTG indicates a permanent damage to the vessel wall and the myocytes and does not represent endothelial dysfunction. Likewise, absence of dilatation in response to hypoxia induced by sphygmomanometer, but its presence in response to NTG administration indicates the normal functioning of the myocytes of the arteries and hence implies Endothelial Dysfunction.

Advantages of the study

Assessment of endothelial function using FMD technique has numerous advantages. It is simple, economical, non invasive, widely available test, can be used routinely as a surrogate marker for atherosclerosis, easy to perform, correlates with the duration of diabetes and the macro-vascular disease, furthermore and FMD offers an assessment of endothelial function in conduit arteries, which are the vessels prone to the development of atherosclerosis.

More number of studies using a large patient population and comparing FMD with carotid intimal and medial thickness will be rewarding for the more accurate measurement of Endothelial Dysfunction.

ADVANTAGES OF MEASURING BRACHIAL ARTERY FMD

Currently, there is ample evidence to suggest that endothelial dysfunction of the coronary circulation is a systemic disturbance of vasculature, which occurs simultaneously in other vascular territories. Several studies have addressed the issue that brachial artery FMD measurement may represent a surrogate marker for diagnostic evaluation of coronary circulation in patients with CAD or those at risk for CAD. The impaired brachial artery FMD not only coincides but also correlates with a greater intima-media thickness of the common carotid artery, indicating early functional and structural changes of the vascular endothelium. Thus, FMD measurement enables us to identify patients at risk for future atherosclerotic complications.
Limitations of the Study

• The above study is a Case-Control study and not a cohort study,

• Long time follow up of the subjects is required to permit a

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statistically confirmed statement with regard to prediction of future atherosclerotic events, based on endothelial dysfunction, which is not available in the above study.

• The major limitation of the present study and several other studies is that the technique is relatively new and can be performed accurately only by few trained personnel.

• The results of the study are highly operator dependent.

• The number of patients taken into the study is only 50 cases and 50 control population were selected. More number of studies with more number of cases and controls are required to generalize the findings to the whole population.

• Some of the cases were on pharmacological treatment for diabetes and some were on ACE inhibitors which can favourably influence endothelial function resulting in false values.

• Other caveats are that arteries smaller than 2.5 mm in diameter are difficult to measure, and vasodilatation is generally less difficult to perceive in vessels larger than 5.0 mm in diameter.

No longitudinal studies in humans have yet proven that these young subjects with endothelial dysfunction will go on to develop advanced atherosclerosis, since such studies would take decades to complete. Despite this, endothelial dysfunction is spatially and temporally linked to atherosclerosis, occurring first at coronary branch points where plaques tend to develop [7] and preceding occlusive arterial disease [8]. Thus, there are much data to support a link between arterial endothelial dysfunction and later advanced atherosclerotic disease and the use of FMD as a surrogate end-point in clinical trials appears justified. Therefore, it is currently recommend that these techniques be used in clinical research, rather than in routine clinical practice. Future developments are likely to enhance the image quality during peripheral arterial scanning, to automate and improve analytical methods for measuring arterial diameter [10] and to link abnormalities observed using this method with cardiovascular event rates.

Suggestions for the future:

• The present study findings may have implications about the origins of vascular disease in type 2 diabetes as well as patients with dyslipidemia.

• The study results emphasize the importance of early detection and control of vascular risk factors in type 2 diabetes, because these people are at a particular risk for developing early structural atherosclerotic changes.

• The ultrasound assessment of arterial FMD responses might provide a valuable tool for risk stratification of patients with type 2 diabetes.

• Improved techniques of FMD measurements will enable clinicians to measure FMD in large-scale trials, thus to further the proof of a relationship between ED and the major clinical endpoints, cardiovascular mortality and morbidity.

• In parallel, new developments in the field of DNA array technologies will help to identify target genes important for different phases of atherosclerosis. These developments may replace mere angiographic detection of vascular disease to the more functional and genomic approach.

CONCLUSION

In Type 2 Diabetes, Endothelial Dysfunction occurs long before the formation of structural atherosclerotic changes, and plays a key role in the etiopathology of the vasculopathy associated with this disease. Endothelial function is known to depend on the ability of endothelium to release NO, in response to shear stress. Assessment of endothelial function can be used as an early marker of future atherosclerotic disease. Given the paucity of facilities and financial constraints for evaluation of endothelial function in India, measurement of endothelial dysfunction using a non-invasive method like brachial artery FMD acts as an attractive prospect. Longitudinal studies involving a large number of people are required to demonstrate the utility of this test as a predictor of atherosclerosis in diabetes in Indian population.

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