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Original Research Article

Comparison of degree of endothelial dysfunction in diabetic patients with and without dyslipidemia

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ABSTRACT

Background: Atherosclerosis in patients with type 2 diabetes is multifactorial. Among other factors, dyslipidemia and increased levels of oxidized LDL are important pathogenic mechanisms of endothelial dysfunction in patients with diabetes. Non-invasive method of assessment of endothelial function by brachial artery flow-mediated vasodilatation (FMD), provided an extremely useful tool for clinical application.
Materials and Methods: 90 patients attending the medicine and endocrinology departments were included in the study. Informed consent was obtained from all the study subjects. Clinical examination: Blood pressure (BP) and body-mass index (BMI); biochemical assessment, which included fasting blood sugar (FBS) and post-prandial blood sugar levels; and comprehensive lipid profile. The brachial artery diameter was measured on B-mode ultrasound images. FMD was calculated. Severe endothelial dysfunction was defined as FMD < 4.5%, as has been described. Data were collected and managed on an Excel worksheet.
Results: The average FMD among controls was 14.76±2.17%, and among diabetics was 7.17±5.1%. Within the dyslipidemia subgroup, FMD was markedly reduced with an average dilatation of 5.74±5.02. Severe endothelial dysfunction was prevalent in 26% of the diabetics. Endothelial dysfunction could not be demonstrated in controls. Among patients with diabetic dyslipidemia, the prevalence of ED was 41%.
Conclusion: The present study findings may have implications about the origins of vascular disease in type 2 diabetes as well as patients with dyslipidemia. The ultrasound assessment of arterial FMD responses might provide a valuable tool for risk stratification of patients with type 2 diabetes, especially for those with dyslipidemia.

1. Introduction

Diabetes mellitus is a serious global health problem and is expected to become worse. The frequencies of coronary heart disease, stroke and peripheral vascular disease are all about 2-4% higher in either type 1 or in type 2 diabetes compared with the general population.[1] Atherosclerosis in patients with type 2 diabetes is multifactorial and includes a very complex interaction, including hyperglycemia, hyperlipidemia, oxidative stress, accelerated aging, hyperinsulinemia, and/or hyperproinsulinemia and alterations in coagulation and fibrinolysis.[2–4] A current hypothesis for the initial lesion in atherosclerosis involves changes in the endothelial cell function. 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

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precluded their use in clinical practice. The development of a non-invasive method of assessment of endothelial function by brachial artery flow-mediated vasodilatation (FMD), as described by Celermajer, provided an extremely useful tool for cardiovascular research and for clinical application.[5–7] This test can be performed easily and has proven reproducibility.[8]

Among other factors, dyslipidemia and increased levels of oxidized LDL are important pathogenic mechanisms of endothelial dysfunction in patients with diabetes. Since it has been shown that treatment of dyslipidemia with drugs improves endothelial function in non-diabetic, dyslipidemic individuals, it becomes very tempting to extrapolate the conclusions from these findings to the diabetic population.[9–11]

In view of the above-stated facts, the present study aims to demonstrate and determine whether impairment in endothelial function occurs in patients with type 2 diabetes mellitus. It also aims to determine whether there is an increased dysfunction of the endothelium in the presence of diabetes mellitus with dyslipidemia.

2. Materials and Methods

90 patients attending the medicine and endocrinology departments at KGH during the period from Sep 2009 to Oct 2010 were included in the study. Inclusion criteria include patients with Diabetes mellitus diagnosed by the ADA criteria and patients with dyslipidemia categorized as per the NCEP ATP-III guidelines. Informed consent was obtained from all the study subjects. Patients with the presence of clinically evident micro/macrovascular complications of diabetes have been excluded. The presence of clinically evident micro/ macrovascular complications was excluded by using clinical or ECG evidence of CAD, past or present history of stroke or TIA, clinical evidence of peripheral vascular disease, features suggestive of diabetic retinopathy on fundus examination, presence of overt proteinuria or serum creatinine > 2mg/dL and clinical evidence of diabetic neuropathy.

Clinical examination included blood pressure measurement, cardiovascular examination, and body mass index (BMI). Plasma glucose, serum cholesterol, serum triglycerides, and high-density lipoprotein-cholesterol (HDL-C) were estimated. LDL-cholesterol (LDL-c) was calculated using the Friedewald formula.

The brachial artery diameter was measured on B-mode ultrasound images. The right brachial artery was studied in all the subjects. A resting scan was obtained, and the velocity of arterial flow was measured with a pulsed Doppler signal at a 700 angle to the vessel, with the range gate (1.5 mm) in the center of the artery. Increased flow was then induced by the inflation of a sphygmomanometer cuff placed around the forearm (distal to the scanned part of the artery) to a pressure of 200 mmHg for 4.5 minutes, followed by release. A second scan was performed continuously for 30 seconds before and 90 seconds after the deflation of the cuff, including a repeated recording of flow velocity for the first 15 seconds after the cuff was released.

FMD was calculated, and the average results of the two observations were recorded. Flow-mediated dilatation was presented as the percent change from baseline to hyperemia. Severe endothelial dysfunction was defined as FMD < 4.5%, as has been described.

2.1. Calculation of FMD

FMD = (d2-d1) x 100/d1

Where,

d1 is the brachial artery diameter at baseline

d2 is the brachial artery diameter at 1 minute of cuff release

Reactive hyperemia was established by an increase in peak systolic velocity (PSV) from baseline to immediately after cuff release.

2.2. Statistical analysis

Data were collected and managed on an Excel worksheet. All values are expressed as mean ± SD. The unpaired Student's t-test was used to compare the mean of continuous variables. The correlation coefficient was used to determine if an association existed among diabetes, dyslipidemia, and endothelial function. P values ≤ 0.05 were considered significant.

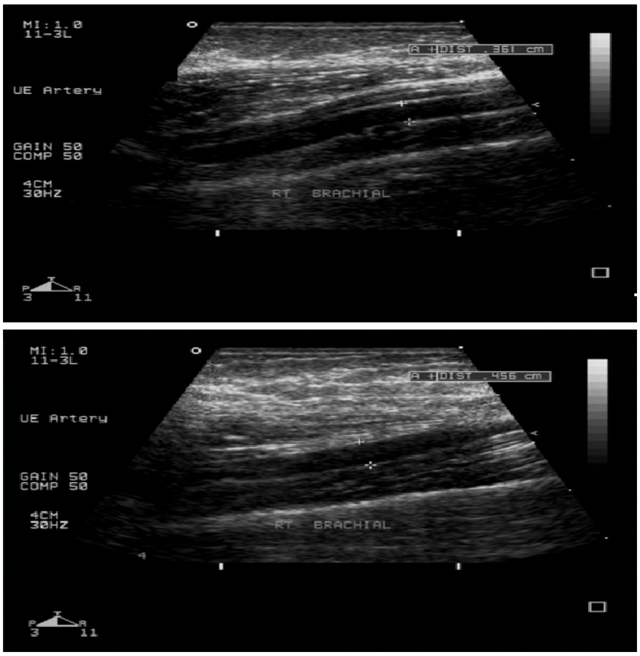

Figure 1: Normal dilatation of brachial artery from baseline to 90 sec after cuff release

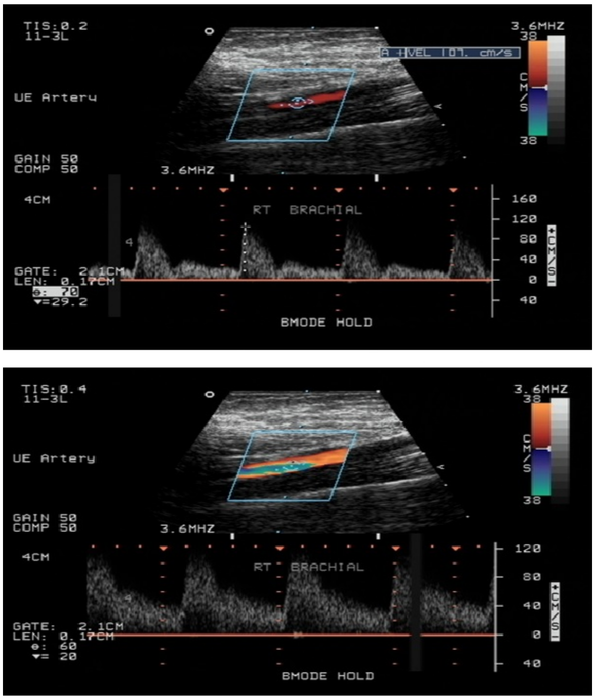

Figure 2: Presence of reactive hyperemia as shown by the increase in peak systolic velocity

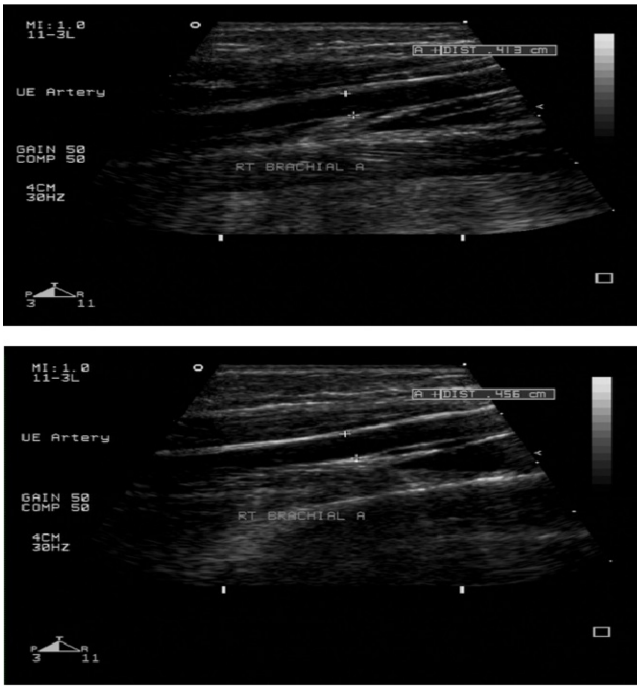

Figure 3: Increased diameter of brachial artery from baseline to 90 sec after cuff release

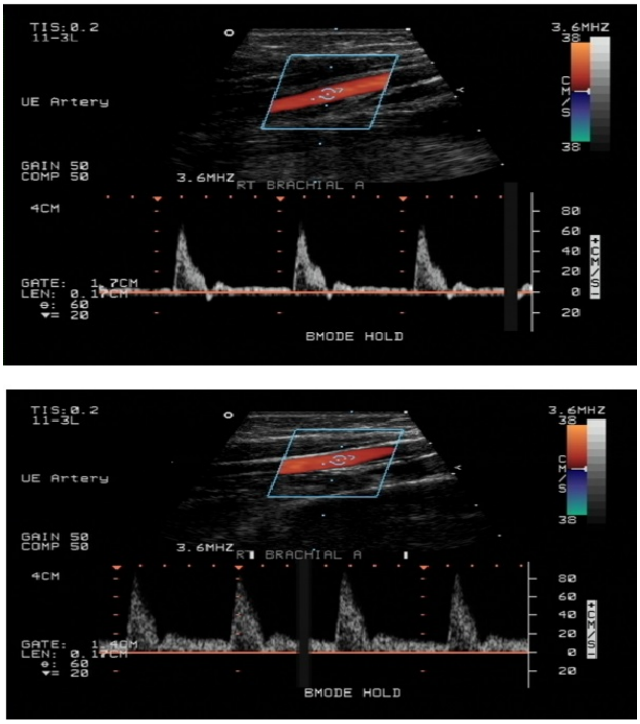

Figure 4: Presence of reactive hyperemia as shown by the increase in peak systolic velocity

Figure 5: Complete absence of dilatation of brachial artery from baseline to 90 sec after cuff release

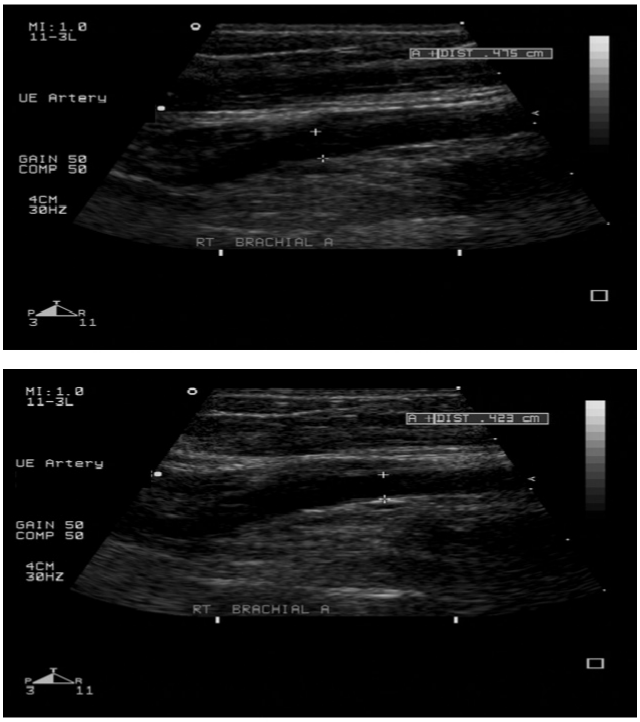

Figure 6: Complete absence of dilatation of brachial artery from baseline to 90 sec after cuff release

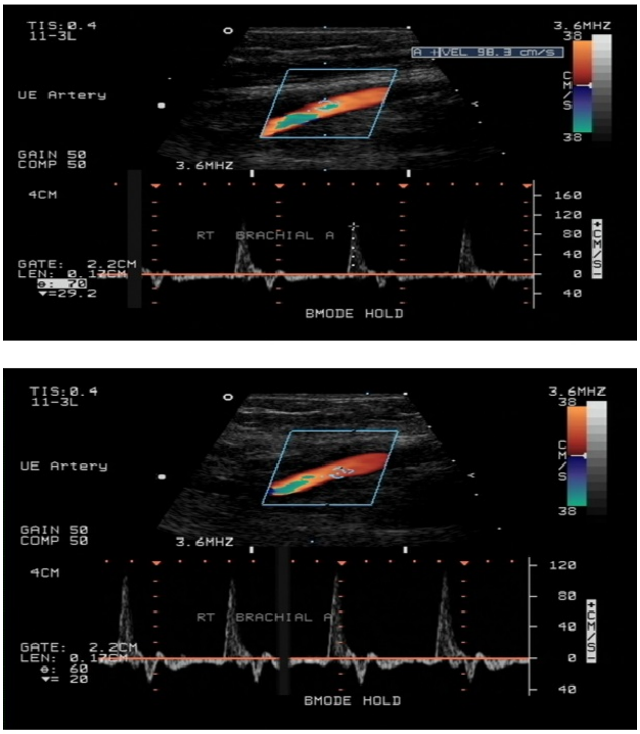

Figure 7: Presence of reactive hyperemia as shown by the increase in PSV

3. Results

In the present study, 30 controls and 60 cases were taken. Cases were further stratified into two groups. Diabetics with dyslipidemia were one group (*n*=34), and those without dyslipidemia were the other group (*n*=26). The presence of diabetes mellitus, as diagnosed by the ADA criteria. Presence of dyslipidemia as per the NCEP ATP–III guidelines.

Table 1: Age distribution of cases

	31-40 yr	41-50 yr	51-60 yr	61-70 yr
Males	2	14	18	2
Females	2	8	14	0

Table 2: Age distribution of controls

	41-50 yr	51-60 yr
Males	8	10
Females	2	10

Most of the subjects are in the 51-60 years age group. Controls were 20 (66.67%), and cases were 32 (53.33%).

Table 3: Baseline clinical characteristics in the study population

S. No.	Variable	Controls	Cases	*p*-value
1	Age	51.6±3.43	51.13±5.48	<0.7
2	BMI	23.52±3.16	24.55±3.93	<0.3
3	TC	172.26±20.00	191.13±44.63	<0.05
4	HDL-C	45.9±5.33	43.06±9.96	<0.21
5	LDL-C	92.26±17.35	120.03±35.76	<0.0001
6	TG	170.26±17.35	164.26±83.0	<0.7
7	TC/HDL	3.76±0.43	4.81±2.18	<0.01

BMI: Body mass index, TC: Total cholesterol, HDL: High density lipoprotein, LDL-c: Low density lipoprotein cholesterol, TG: Triglycerides.

A baseline comparison of lipid profiles between the diabetes group and the control group showed 1) elevated BMI, 2) elevated total cholesterol, 3) lowered HDL-C, 4) elevated LDL-C, and 5) lowered TG in the diabetes group. Of these, the increase in TC and LDL was found to be statistically significant.

When a comparison was made between diabetes with dyslipidemia and diabetes without dyslipidemia, the following differences were observed:

1. The average age of the dyslipidemia group was lower.
2. They had a higher body mass index.
3. There was an elevated TC, elevated LDL-C, TG, TC/HDL-C, and decreased HDL-C levels.
4. All the values were statistically significant.

The average FMD among controls was 14.76±2.17%, and among diabetics was 7.17±5.1%. Within the

Table 4: Comparison of lipid profile between diabetics with and without dyslipidemia

S. No.	Variable	Diabetes without dyslipidemia	Diabetes with dyslipidemia	*p*-value
1	Age	53.61±5.51	49.23±4.77	<0.03
2	BMI	21.61±3.01	26.79±2.99	<0.0001
3	TC	168.92±33.76	208.11±45.25	<0.01
4	HDL-C	50.46±5.09	37.41±9.06	<0.001
5	LDL-C	99.30±22.41	135.88±36.41	<0.002
6	TG	92.23±21.97	219.36±70.56	<0.0001
7	TC/HDL	3.35±0.61	5.74±2.3	<0.0002

BMI: Body mass index, TC: Total cholesterol, HDL: High density lipoprotein, LDL-c: Low density lipoprotein cholesterol, TG: Triglycerides.

Table 5: Comparison of FMD between cases and controls

S. No.	Variable	Controls	Cases	*p* -value
1	FMD	14.76±2.17	7.17±5.1	<0.0001

Table 6: Comparison of FMD between diabetes with and without dyslipidemia

S. No.	Variable	Diabetes without dyslipidemia	Diabetes with dyslipidemia	*p*-value
1	FMD	9.82±4.39	5.74±5.02	<0.02

dyslipidemia subgroup, FMD was markedly reduced with an average dilatation of 5.74±5.02.

Table 7: Prevalence of endothelial dysfunction in the cases and controls

Variable	Controls (*n*=30)	Cases (*n*=60)	Normal lipid profile (*n*=26)	Dyslipidemia group (*n*=34)
FMD <4.5	0 (0%)	16 (26%)	2 (14%)	14 (41%)
FMD >4.5	30 (100%)	44 (74%)	24 (92%)	20 (59%)

Severe endothelial dysfunction was prevalent in 26% of the diabetics. Endothelial dysfunction could not be demonstrated in controls. Among patients with diabetic dyslipidemia, the prevalence of ED was 41%.

A significant negative correlation is seen between age, BMI, TC, LDL-C, TG, and the extent of endothelial dysfunction.

Table 8: Correlation of FMD with diabetic dyslipidemia on univariate analysis

S. No.	Variable	*r*-value	*p*-value
1	AGE	-0.54	>0.001
2	BMI	-0.43	>0.0001
3	TC	-0.37	>0.005
4	HDL	+0.0001	Not significant
5	LDL	-0.32	>0.05
6	TG	-0.63	>0.0001
7	TC/HDL	-0.25	Not significant

BMI: Body mass index, TC: Total cholesterol, HDL: High density lipoprotein, LDL-c= Low density lipoprotein cholesterol, TG: Triglycerides.

4. Discussion

In the present study, there was an impairment of flow-mediated dilatation in diabetics when compared with normal subjects (7.17±5.1 vs. 14.76±2.17, *p*-value <0.0001). Within the diabetes group, patients with dyslipidemia had shown significant impairment in FMD than patients without dyslipidemia (5.74±5.02 vs. 9.82±4.39, *p*-value <0.02). A significant negative correlation was found between TC, LDL-C, TG, and TC/HDL ratio and the degree of impairment of endothelial function.

Similar results were obtained in other studies. In a study by K. Bhargava *et al.*, significant endothelial dysfunction was observed in diabetes (5.51±2.21%) when compared with normal subjects (7.03±2.87%).[12] 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.*, significant impairment of endothelial function occurred in diabetics when compared with non-diabetics (*p*-value <0.05).[13] They also showed that endothelial dysfunction was more prevalent in the dyslipidemia group. 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).[14–17] 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.[18]

Yerong Yu *et al.* have shown that compared with type 2 diabetes patients with normoalbuminuria, patients with microalbuminuria had more prominent endothelial dysfunction (9.7±4.3 vs. 8.0±3.8%, *p*-value <0.05).[19] Antonaides *et al.* 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).[20] They have also shown that vitamin C, which acts as an antioxidant, has improved the forearm vasodilatory response.

4.1. Advantages of the study

Assessment of endothelial function using the FMD technique is a simple, economical, noninvasive, widely available test that can be used for both diagnostic and therapeutic purposes. However, the major limitation of the present study is that the technique is relatively new, and the patients taken into each study are few in number.

4.2. Limitations of the study

Though low HDL level in diabetes, which is proven to cause endothelial dysfunction, was associated with impairment of endothelial function in this study, it did not show a statistically significant independent effect. This may be due to the small sample size. This is not a cohort study and a long follow-up of the subjects is required to permit a statistically confirmed statement regarding the prediction of future atherosclerotic events based on endothelial dysfunction.

Some of the cases were on pharmacological treatment for diabetes and dyslipidemia with drugs that are proven to improve endothelial function (e.g., Metformin, Statins). This could be a confounding factor in the present study.

4.3. Suggestions for the future

The study results emphasize the importance of early detection and control of vascular risk factors in type 2 diabetes because these people are at 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, especially for those with dyslipidemia.[21,22] 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 with a more functional and genomic approach with the perspectives to evaluate new strategies in risk stratification and treatment.

5. Summary and Conclusions

The development of the non-invasive method of endothelial function assessment by flow-mediated vasodilatation (FMD) provided an extremely useful tool for cardiovascular research and for clinical application. A close correlation of endothelial function in the human coronary and peripheral vasculature has been demonstrated. Ultrasonography is a reliable and accurate technique to determine flow-mediated dilatation in the superficial arteries. Reproducibility of FMD determination is best in the brachial artery in healthy subjects and in patients with atherosclerosis. B-mode ultrasound scan, including brachial artery FMD, may be of clinical value in the screening for endothelial dysfunction.

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.

6. Source of Funding

None.

7. Conflict of Interest

None.

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