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Research Article

Assessment of endothelial dysfunction in diabetes mellitus in Indian population by color Doppler

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ABSTRACT

Background: Endothelial dysfunction is regarded as a systemic marker of cardiovascular disease. Brachial artery flow-mediated dilation (FMD) is a mode of evaluating endothelial function and for early diagnosis of atherosclerotic diseases. We studied whether there is a difference in vascular endothelial function between type 1 and 2 diabetes mellitus and normal population.
Methods: We assessed %FMD of 50 patients with diabetes mellitus and 50 control populations without diabetes mellitus or other risk factors. SPSSS version 13 was used for statistical analysis. Students T test was used for comparison of means of the two groups.
Results: The %FMD was significantly lower in patients with type1 and 2 diabetes mellitus compared to normal population.
Conclusions: Diabetes mellitus is associated with endothelial dysfunction irrespective of Type 1 or Type 2 diabetes mellitus, as suggested by impairment in vascular reactivity to hyperemia in both. Monitoring FMD may help in assessment of progression of atherosclerosis.

Keywords: Diabetes mellitus, Endothelial dysfunction, FMD, Atherosclerosis

INTRODUCTION

Diabetes mellitus is a heterogeneous group of metabolic disorders characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both.[1]

Adults with diabetes are at a 2 to 4 fold increased risk of cardiovascular events relative to those without diabetes.[2] Cardiovascular disease accounts for up to 80% of premature excess mortality in diabetic patients.[3] Endothelial dysfunction is regarded as a systemic marker of cardiovascular disease. Impaired arterial vasodilation can lead to thrombosis or other precursors of atherosclerotic lesions.

When endothelial dysfunction exists it is not limited to areas of atherosclerosis so that dysfunction in the brachial artery, for example, indicates dysfunction throughout the vascular system, including the coronary arteries.[4]

Endothelial dysfunction is caused by risk factors, which have basic and initial role in atherogenesis. During further progression it is still not known whether and how is endothelial dysfunction influenced by the risk factors in the late phase of atherosclerosis and / or what kind of other factors play role in endothelial dysfunction.[5]

METHODS

The present case control study was undertaken in the Department of Medicine in the NSCB Medical College and Hospital, Jabalpur, Madhya Pradesh. The study period was from September 2006 to September 2007. The study included 50 subjects who were diabetes mellitus patients and 50 control (normal subjects), who had no history of diabetes mellitus belonging to same age group.

Inclusion criteria

- Age group 20-60 years.
- Diabetes mellitus patients diagnosed by W.H.O criteria of:

a) FBS≥126mg% or
b) Symptoms of diabetes mellitus with RBS≥ 200mg% or
c) 2 hours plasma glucose ≥200mg% in oral GTT

- Subjects who wanted to participate in the study.

Exclusion criteria

- Diabetes mellitus patients with complications of infection, diabetic ketoacidosis, hyperglycemic hyperosmolar state and with chronic microvascular and macrovascular complications of retinopathy, overt nephropathy, CAD, stroke, peripheral vascular disease.
- Subjects not fulfilling inclusion criteria were excluded from the study.
- History of systemic HTN
- Congestive cardiac failure
- Renal insufficiency
- Patient with dyslipidemia
- History of smoking.
- Postmenopausal females.

Complete clinical history including age, sex, symptoms, duration of symptoms, family history and treatment was taken. Complete clinical examination including blood pressure measurement, cardiovascular examination, ECG, anthropometric measurement, body mass index (BMI), fundus examination, biochemical assessments include blood sugar (fasting blood sugar (FBS), post prandial blood sugar (PPBS).

Lipid profile, CBC with ESR, renal function test (serum electrolytes, Blood urea, serum creatinine, urine albumin, sugar) and endothelial function of subjects assessed by color Doppler of brachial artery (B mode ultrasonography for flow mediated vasodilatation of brachial artery).

Study protocol

Each subject made one visit to the study hospital during which medical history, physical examination and systemic examination was done. Height, weight and blood pressure were measured. Fasting blood sample was taken for lipid profile and blood sugar. Blood and urine samples were tested for remaining biochemical analysis.

Measurement of biochemical parameters

Folin Wu method was used for the measurement of blood sugar. For estimation of serum lipids subjects were requested to fast for 12 hours overnight. Fasting venous blood samples were collected in sitting position in plain bulbs. Fasting serum lipid profile estimation was done by enzymatic method using dialab kits. Samples were processed on EKBA CHEM - 5 plus semiautoanalyser of TRANSASIA.

For estimation of total serum cholesterol enzymatic method of CHOD-PAP was used. Normal range of serum cholesterol is <200mg/ dL. For estimation of serum triglycerides enzymatic method of GPO-PAP was used in which also the H2O2 produced by serial reaction is measured enzymatically. Normal range of serum triglycerides<160 mg/ dL.

Doppler study of the brachial artery

The ultrasound procedure done in supine position at the room temperature. High resolution Siemens echo-Doppler with 7.5 MHz high frequency linear vascular probe that has an axial resolution of 0.1 mm was used for small part of brachial artery to measure arterial diameter of the right brachial artery at a fixed position about 5 cm above the elbow joints.

The subjects are allowed to rest for at least 10 minutes before the first scan; the measurement will be performed basally at rest, during reacting hyperemia. Arterial diameter (distance between the M-lines) of proximal and the distal wall i.e. the interface between the tunica media and adventitia; which is easier to detect than the surface of the endothelial layer will be measured on a two dimensional ultrasonic calipers and the smallest diameter will be taken.

First lumen diameter of the brachial artery will be searched in a cross sectional view and then measured in a longitudinal section by B-mode. Two focus zones set to the depth of the transducer near wall and far wall respectively. Besides optimizing depth and gain setting individually, a present vascular imaging program will be used to standardize the measurements.

After 10 minutes rest, the first scan will be taken, after that ischemia will be induced by inflation of pneumatic tourniquet (sphygmomanometer cuff) at the ipsilateral forearm to a pressure to 200 mmHg for 5 minutes.

Followed by sudden deflation to generate an increase in blood flow in the brachial artery located proximal to the tourniquet. Lumen diameter will be measured 90 seconds after deflation of cuff.

Percentage increase in lumen diameter during post ischemic hyperemia as compared to basal lumen diameter will be labeled as flow mediated dilatation (FMD%) a marker of endothelium dependent dilation.

Statistical analysis

Data were statistically analyzed using SPSS version 13 (SPSS, Chicago, IL, USA). For comparison of FMD% of the groups and for comparing background characteristics and results of blood chemistry tests, Student's t-test was carried out.

RESULTS

In this case control study 50 North Indian patients of diabetes mellitus were selected from both outpatients and inpatients of N.S.C.B Medical College, Jabalpur, Madhya Pradesh, India and 50 otherwise normal persons were studied on same lines and they served as controls. From table 1 it is seen that within the case group of 50 patients 31 patients are males and 19 are females. The control group has 25 males and 25 females. The mean age of male cases is 42.84±9.21 and mean age of male controls is 40.28±10.51 (p value>0.05).

Table 1: Age and sex group.

Age group	Case			Control		
	Male	Female	Total	Male	Female	Total
20-29	4 (12.9%)	2 (10.5%)	6 (12.0%)	5 (20.0%)	5 (20.0%)	10 (20.0%)
30-39	6 (19.4%)	5 (26.3%)	11 (22.0%)	8 (32.0%)	8 (32.0%)	16 (32.0%)
40-49	13 (41.9%)	8 (42.1%)	21 (42.0%)	6 (24.0%)	6 (24.0%)	12 (24.0%)
50-59	8 (25.8%)	4 (21.1%)	12 (24.0%)	6 (24.0%)	6 (24.0%)	12 (24.0%)
Total	31	19	50	25	25	50
Mean±SD	42.84 (9.21)	40.79 (8.43)	-	40.28 (10.51)	39.52 (10.23)	

Table 2: Baseline diameter of brachial artery.

Group	Mean	SD	N	Minimum	Maximum
Case	3.438	0.3368	50	2.8	4.3
Control	3.452	0.3996	50	2.9	5.0

The mean age of female cases is 40.79±8.43 and mean age of female controls is 39.52±10.23 (p value>0.05). The baseline diameter of brachial artery is depicted in table 2 and it shows that mean diameter of brachial artery in case group is 3.438±0.3368 mm and in control group 3.452±0.3996 mm. p value>0.05.

Table 3: Post hyperemia diameter of brachial artery.

Group	Mean	SD	N	Minimum	Maximum
Case	3.700	0.3344	50	3.1	4.6
Control	3.940	0.4111	50	3.4	5.5

Table 4: Change in diameter of brachial artery.

Group	Mean	SD	N	Minimum	Maximum
Case	0.26	0.1214	50	0	0.5
control	0.48	0.1060	50	0.2	0.8

The post hyperemia diameter is shown in table 3 and in case group it is 3.700±0.3344 mm and in control group it is 3.940±0.4111 mm. P value<0.005. The table 4 shows that mean change in brachial artery diameter is 0.26±0.1mm in case group compared to 0.4±0.1mm in control group (p value<0.0001).

Table 5: Flow mediated vasodialatation (FMD%).

Group	Mean	SD	N	Minimum	Maximum
Case	7.765	3.8155	50	0	16.2
Control	14.313	3.3915	50	5.9 1	21.2

Table 6: FMD% with sex group.

Group	Sex	Mean	SD	N	Minimum	Maximum
Case	Male	7.838	4.0647	31	0	16.2
	Female	7.647	3.4741	19	2.8	14.3
Control	Male	13.141	3.7932	25	5.9	21.2
	Female	15.485	2.4969	25	10.8	20.0

From the Table 5 it is seen that mean FMD% in Diabetic cases is 7.765±3.8155% as compared to FMD% in controls of 14.313 ± 3.3915% (p value<0.0001). From the table 6 we can see the mean FMD% in male cases is 7.838±4.0647% and male controls is 13.141±3.7932% (P value<0.0001). The mean FMD% in female cases is 7.647±3.4741% and in female controls is 15.485±2.4969% (P value <0.0001).

Table 7: FMD% and age group.

Group	Age group	Mean	SD	N	Minimum	Maximum
Case	20-29	9.315	4.5566	6	2.9	16.1
	30-39	9.519	2.3612	11	6.2	14.3
	40-49	7.228	3.2671	21	2.8	16.2
	50-59	6.324	4.8713	12	0.0	15.2
Control	20-29	14.733	2.9926	10	10.0	20.0
	30-39	14.213	3.4778	16	8.1	21.2
	40-49	13.696	2.3884	12	10.8	19.0
	50-59	12.713	4.0816	12	5.9	19.4

Table 8: FMD% *group* type of DM.

Group	Type DM	Mean	SD	N	Minimum	Maximum
Case	Type-I	8.916	4.0202	8	2.9	16.1
	Type-II	7.546	3.7857	42	0.0	16.2
Control		14.313	3.3915	50	5.9	21.2

The FMD % between males and females in both case and control group has p value >0.05. Table 7 shows that in the Age group 20-29 mean FMD % in case group is 9.315±4.5566% compared to 14.733±2.9926% of control group (p value<0.01). Age group 30-39 have mean FMD % in case group 9.519±2.3612% compared to 14.213±3.4778% in control group (P value<0.05). Age group 40-49 have mean FMD % in case group 7.228±3.2671% compared to 13.696±2.3884% of control group (p value <0.05). Age group 50-59 have FMD % in case group 6.324±4.8713% compared to 12.713±4.0816% in control group (P value<0.001).

From the Table 8 we see cases of type 1 diabetes mellitus were total 8 in number and have mean FMD% 8.916±4.0202% (p value<0.001). Cases of type 2 diabetes mellitus were 42 in number and have mean FMD% 7.546±3.7857% (p value< 0.0001). The controls were total 50 and have mean FMD% of 14.313±3.3915%. FMD% between type 1 and type 2 diabetes mellitus (p value>0.05).

DISCUSSION

Endothelial dysfunction occurs early in atherosclerosis, predating clinical disease. Endothelial dysfunction in diabetes occurs very early. Non-invasive method of flow mediated vasodilatation might be useful in clinical practice for early diagnosis of subclinical atherosclerosis, which can allow for strategies designed to reduce

cardiovascular event rate in these patients. The mean change in diameter of brachial artery in case group was 0.26±1.12 mm. The mean change in diameter of control group was 0.48±0.10mm. There is a significant difference in the change in diameter of two groups as P value<0.0001. The mean FMD% of case group was 7.7±3.8%. The mean FMD% of control group was 14.3±3.3%. There is a very significant difference in FMD% of two groups as P value <0.0001. Thus FMD% is significantly lower in diabetes mellitus patients compared to normal subjects suggestive of endothelial dysfunction in diabetes mellitus. In Hoorn study by Henry RM, Stehouwer CD et al, a population-based study involving (n = 650); 246 with normal glucose metabolism (NGM), 135 with IGM (impaired glucose metabolism) and 269 with DM2 (type 2 diabetes mellitus).

Table 9: FMD studies in diabetes mellitus type 1 and type 2.

Reference	Subjects	FMD% cases	FMD % controls	P value	Type
Dogra et al[11]	17 cases 17 controls	5.4±0.6	7.9±0.6	<0.001	Type 1
Clarkson et al[12]	80 cases 80 controls	5±3.7%	9.3±3.8	<0.001	Type 1
Enderle et al[13]	25 cases 25 controls	3.8±3.3	6.9±4.4	<0.01	Type 2
Poredos et al[14]	38 cases 35 controls	4.15±2.8	11.3±3.6	<0.0001	Type 1
Vasilescu et al[15]	10 cases 10 controls	5.6±1.4	7.1±1.0	<0.01	Type 2
Lekakis et al[16]	26 cases 26 controls	5.8±7.0	11±7	<0.01	Type 1

The increase in brachial artery diameter (mean±standard deviation) in NGM, IGM and DM2 was 0.19±0.15, 0.19±0.18 mm, 0.13±0.17. IGM was not associated with impaired FMD. Additional adjustment for conventional cardiovascular risk factors did not alter these associations. Hyperglycemia or hyperinsulinemia explained 2% of the association between DM2 and FMD. This study shows that DM2 is independently associated with impaired FMD.

Hyperglycemia and hyperinsulinemia contribute minimally to this association. Impaired FMD may therefore, in part, explain the increased cardiovascular disease risk in DM2 whereas the normal FMD in IGM suggests that other forms of endothelial dysfunction are important in explaining the increased cardiovascular disease risk in IGM.[6]

Nair BM et al in their study did flow mediated dilatation (FMD) determined using high resolution ultrasonography in 20 non-diabetic subjects and in 23 type 2 diabetic subjects without any complications showed age-adjusted mean (S.D.) FMD value in diabetic subjects (8,9±5%) was lower (P < 0.0001) when compared with the group of control subjects (18.8±7.5%).[7]

A similar study was carried out by Kam S. Woo, Celermajer et al in Chinese University of Hong Kong in China. Atherosclerotic disease is much less common in Chinese subjects compared with Caucasian subjects. They studied arterial endothelial function in 36 asymptomatic Chinese adults (age 29±6 years).

Of these subjects, 18 had type1 diabetes and 18 were age- and sex-matched normoglycemic control subjects. No subjects in either group had clinical evidence of atherosclerosis, and none were taking any lipid-lowering drugs or cardiovascular medications. There were no significant differences between the two groups in anthropometric parameters, blood pressure, or lipid profile. The baseline flow and vessel size were similar between both groups. The diabetic group had lower FMD (6.8±2.2 vs. 9.1±2.0%, P-0.003 compared with the control subjects.

The mean FMD % in case group with type 1 diabetes mellitus (n=8) is 8.9 ±4.0%.The mean FMD% type 2 diabetes mellitus (n=42) case was 7.5±3.7%. The mean FMD % of type 1 and type 2 diabetes mellitus was significantly lower than control group (n=50) FMD% of 14.3±3.3% as p value was <0.001 and <0.0001 respectively. In the study there was no significant difference in FMD% in type l and type 2 diabetes mellitus as p value was >0.05.[8]

Balletshofer BM et al examined peripheral endothelial function in a group of young insulin resistant subjects in a cross- sectional study and compared these results with a metabolically healthy (insulin sensitive) control group. A marked reduction in flow-mediated vasodilation in insulin resistant (IR) subjects (FMD: median 3.4%, range -4.0 to 12.5 in IR versus 6.6%, range -1.2 to 20.1% in insulin sensitive subjects; P = 0.017). They suggested that ED can be detected very early in the life of insulin resistant subjects whereas no significant structural changes, indicated by a thickening of the intima-media layer, could be found.[9]

Beckman TA et al at Brigham and Women's Hospital examined the relationship between insulin resistance and vascular function in three insulin-resistant states (type 2 diabetes, non-HIV lipodystrophic diabetes, and nondiabetic polycystic ovary syndrome [PCOS]) and in healthy control subjects.

Type 2 diabetic, liposdystrophic, and PCOS subjects were insulin resistant compared with control subjects. Flow-mediated vasodilation was reduced in diabetic (3.4±1.3%) compared with control (7.3±1.1%) subjects but not in lipodystrophic (7.7±1.2%) or PCOS (9.9±0.7%) subjects (P = 0.005).

Among these different types of patients with insulin resistance they found abnormal endothelium-dependent vasodilation only in the patients with type 2 diabetes. They concluded that variations in the mechanism of insulin resistance may affect endothelial function differently than glucose homeostasis.[10] Table 9 shows other studies which showed FMD % is lowered in diabetes mellitus Type 1 and Type 2. FMD % was comparable between male and female groups in both case and control groups. Masayoshi Hashimoto et al studied modulation of flow mediated vasodilatation of the brachial artery by sex and menstrual cycle. They found that in females FMD% was highest during follicular phase of menstrual cycle, when serum estradiol was highest. Female FMD% during menstrual phase with low estradiol was comparable to male FMD%.[17]

FMD % progressively decreased with age in both case and control groups in our study. There was a significant decrease in FMD % between the younger and elderly age groups in both case and control groups irrespective of gender. Jensen-Urstad K et al studied gender difference in age related changes in vascular function. Flow-mediated endothelium-dependent dilatation was similar in men and women being 3.1±2.5% (mean±SD) in men vs. 2.6±2.3% in women.

FMD of the brachial artery was negatively correlated with vessel size in both men and women (P<0.001). Men had larger brachial artery diameter than women (4.6±0.7 vs. 3.6±0.4 mm, P<0.001). FMD-induced vasodilatation is smaller in women at 55 years of age than at 35 years of age. FMD was similar in men at 35 and 55 years of age and in men and women at 55 years of age. The smaller FMD in women at 55 years of age, compared with at 35, could be due to postmenopausal hormonal changes.[18]

Maintaining a high level of fitness, or undertaking exercise training, prevents the age-related decline in the brachial artery vasodilator function in women but not in men.[19] In postmenopausal women, endothelial dysfunction was prominent in women with diabetes and was significantly improved by estrogen but not reversed.[20]

So brachial artery flow mediated vasodilatation study done in our population and similar studies in other populations described above prove that endothelial dysfunction in diabetes mellitus can be assessed by flow mediated vasodilatation. Even in diabetic patients endothelial dysfunction can be further affected by several factors like age, gender, glycemic control, exercise training. There is a further need for studying the determinants of endothelial dysfunction in diabetes mellitus and its association with FMD. Thus brachial artery FMD can be a useful tool for assessing the progression of atherosclerosis.

CONCLUSION

Diabetes mellitus is associated with endothelial dysfunction irrespective of Type 1 or Type 2 diabetes mellitus, as suggested by impairment in vascular reactivity to hyperemia in both. Endothelial dysfunction occurs early in diabetes mellitus even before chronic microvascular and macrovascular complications are manifested as this study includes only uncomplicated diabetes mellitus patients.

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