

Association of family history of type 2 diabetes mellitus with markers of endothelial dysfunction in South Indian population

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Studies indicate that risk for type 2 diabetes mellitus (T2D) or cardiovascular disease is detectable in childhood, though these disorders may not emerge until adulthood. This study was aimed to assess the markers of endothelial dysfunction in patients with the family history of T2D from South Indian population. A total of 450 subjects were included in the study comprising Group I (n = 200) of T2D, Group II (n = 200) of age- and sex-matched healthy controls, Group III (n = 25) of children of T2D patients and Group IV (n = 25) of children of healthy controls. Results showed that intimal medial thickening (IMT) was significantly higher in T2D patients, compared with control subjects with no family history of diabetes. The fasting plasma glucose, glycated hemoglobin, serum total cholesterol, triglyceride, LDL-cholesterol, apolipoprotein B (ApoB) and high-sensitive C-reactive protein (hsCRP) levels were significantly increased, whereas HDL-cholesterol and serum nitrite levels were significantly decreased in T2D patients. However, children of T2D patients who were not diabetic did not show significant increase in the IMT, as compared to those of healthy controls. In conclusion, the present study demonstrated that IMT was significantly higher in the T2D patients and increased with age and family history. The increased levels of lipids, hsCRP, IMT and decreased nitrite levels might contribute to the risk of endothelial dysfunction in patients with T2D. However, further studies are warranted with other biomarkers of endothelial dysfunction in T2D patients with increased sample size.

Keywords: Diabetes, Familial history, Diabetic peripheral vasculopathy, Endothelial dysfunction, Intimal medial thickening

Diabetes mellitus (DM) is a heterogeneous disorder characterized by persistent hyperglycemia. Type 2 diabetes (T2D) is characterized by insulin resistance and deficient beta-cell function and usually results from a combination of defects in insulin action and secretion¹. DM and its complications pose a major threat to public health throughout the world. The prevalence of DM in South Indian population is 18.6%². Based on compilation of studies from different parts of the world, the World Health Organization (WHO) has projected maximum increase in diabetes (57 million) in India by 2025. India will continue to have the largest number of diabetic subjects as a result of rapid urbanization and

economic development³. In developing countries, the largest number of people with diabetes is in the age group 40 to 64 yrs, while in developed countries, in those aged 65 yrs and over.

DM is associated with a high risk of cardiovascular disease (CVD) which is the most common cause of mortality in people with diabetes⁴. CVD accounts for more than 80% of deaths in people with diabetes⁵. Thus, in people with diabetes, atherosclerosis not only develops at younger age and it is also more diffuse and severe than that found in people without diabetes. People with diabetes have a two- to four-fold increased risk of peripheral arterial disease⁶. Several studies indicate that risk for T2D or CVD is detectable in childhood, although these disorders may not emerge until adulthood. Hence, examining the family history is the simple way to detect risk for DM or CVD. Epidemiological studies have shown that people with one or more first-degree relatives who are affected with diabetes are 2 to 6-times as likely to have the disease as compared with those having no affected relatives⁷.

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Abbreviations: ApoB, Apolipoprotein B; BMI, body mass index; CVD, cardiovascular disease; DM, diabetes mellitus; HbA1c, glycated hemoglobin; hsCRP, high-sensitive C-reactive protein; IMT, intimal medial thickening; NO, nitric oxide; T2D, type 2 diabetes mellitus.

Apolipoprotein B (ApoB) is a high molecular weight protein that is required for the synthesis and hepatic secretion of very low-density lipoprotein (VLDL). However, recent studies have shown that Apo B provides better information regarding risk of coronary artery disease⁸⁻¹⁰. ApoB identifies high-risk dyslipidaemic phenotypes that are not detected by standard lipid profile in T2D patients¹¹.

Nitric oxide (NO) has emerged as a fundamental signaling molecule, regulating virtually every critical cellular function and is also a potent mediator of cellular damage in many conditions¹². It is synthesized by endothelial cells from L-arginine by endothelial nitric oxide synthase (eNOS). NO is produced from virtually all cell types composing the myocardium and regulates cardiac function through both vascular-dependent and -independent effects. Endothelial dysfunction associated with diabetes has been attributed to lack of bioavailable NO, due to uncoupling of receptor-mediated signal transduction, a deficiency of eNOS substrate L-arginine, or a decreased availability of one or more co-factors essential for optimal functioning of eNOS¹³. Elevated levels of superoxide anion can cause inactivation of NO and impairment of eNOS by triggering advanced glycation end products¹⁴.

Among several markers of inflammation, high-sensitive C-reactive protein (hsCRP), a pentameric protein plays a significant role in DM. It is a non-immunoglobulin protein having five identical subunits. hsCRP is an acute phase response protein which is markedly increased in both inflammatory and infectious diseases¹⁵. Several studies have demonstrated that hsCRP remains a significant predictor of diabetes risk even after adjusting with body mass index, family history of DM, smoking and other factors¹⁶.

The dysfunctional endothelium is characterized by an impaired endothelium-dependent vasodilation response, which favors platelet aggregation and white blood cells (WBCs) adhesion and promotes smooth muscle cell proliferation. Elevation of serum markers (adhesion molecules, selectins, and hsCRP) has been associated with endothelial dysfunction¹⁷. Measurement of carotid IMT is being increasingly used as a non-invasive marker of atherosclerosis¹⁸. Intimal medial thickening (IMT) assessment allows convenient stratification of patients at risk for CVD and has proven to be a good marker. It has been shown that the IMT measurement correlates strongly

with future development of myocardial infarction and stroke^{19,20}.

Since the studies are lacking in India on the influence of family history of T2D with the carotid wall thickening and endothelial function, in this study, we have examined the association of family history of T2D on the markers of endothelial dysfunction in South Indian population. Although several markers have been implicated in endothelial dysfunction, in the current study, we have investigated IMT, hsCRP and nitrite (a stable metabolite of NO) levels in T2D patients and compared with the healthy subjects.

Materials and Methods

Subjects

A total number of 450 subjects from ACS Medical College & Hospital, Chennai were included in the study, i.e., Group I (n = 200) of T2D patients, Group II (n = 200) of age and sex-matched healthy controls, Group III (n = 25) of children of T2D patients, and Group IV (n = 25) of children of healthy controls. The age criteria for the study were in between 21 and 60 yrs, i.e., 31 to 40 yrs of parental generation and 21 to 30 yrs of children. Patients with glycated hemoglobin (HbA1c) 7.0 to 10.0% were enrolled in the study. Patients with history of cardiac, liver, kidney disease, hypertension, cancer, inflammatory diseases and women with oral contraceptives and hormone replacement therapy were excluded from the study. This study was approved by the Institutional Ethics Committee and informed consent was obtained from all the study subjects. The subjects were screened for blood pressure, BMI, family history, medical history and the blood samples were collected for biochemical analysis.

Biochemical analysis

The fasting blood glucose, HbA1c, lipid profile, ApoB and hsCRP levels were measured in T2D patients and controls. The biochemical analysis was done by using commercially available kits (Bayer Diagnostics) on Semi-auto analyzer (Statfax). HbA1c was estimated by ion-exchange resin method (Diatek). The serum hsCRP was estimated by latex turbidimetry method (Euro Diagnostics System). Serum nitrite was determined using the Griess reagent²¹.

IMT measurement

The IMT of carotid artery was determined by using high frequency linear ultrasound (Philips). It was measured as the distance from the leading edge of

the first ecogenic line representing lumen intimal interface to the second ecogenic line representing the collagen containing upper layer of the intimal adventitia²² (Fig. 1).

Statistical analysis

All values were expressed as mean \pm SD. Student's t-test was used to evaluate the differences in continuous variables. Data were analyzed by using Graphpad Prism Software version 6.0. A 'p' value of <0.05 was considered significant.

Results

The physiological and biochemical parameters in cases and controls are shown in Table 1. Results indicated that there was a significant increase in

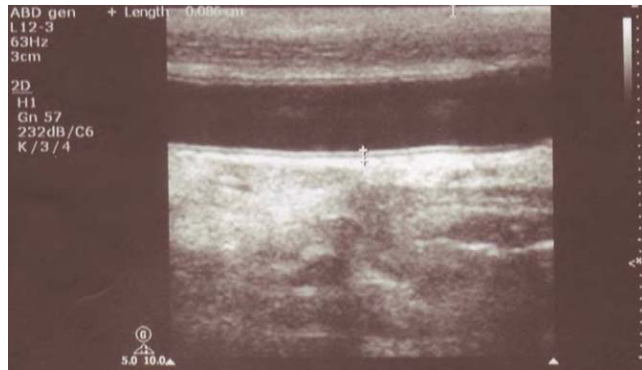


Fig. 1—Common carotid intimal medial thickening (IMT) longitudinal scan determined by ultrasonography

fasting plasma glucose, HbA1c, serum total cholesterol, triglycerides and LDL-cholesterol levels ($p<0.0001$) in T2D patients, as compared to control. The serum HDL-cholesterol and nitrite levels were significantly decreased in T2D patients than controls. The ApoB ($p<0.0001$), hsCRP ($p<0.0001$) levels and IMT ($p<0.0001$) were significantly increased in T2D patients, when compared with healthy controls. No statistically significant differences were observed in fasting plasma glucose, HbA1c, serum total cholesterol, triglycerides, LDL and HDL-cholesterol, ApoB, hsCRP, IMT in children of T2D patients and healthy controls (Table 1).

Among 200 T2D patients, 64 belonged to non-diabetic parents, 81 to single parent diabetic and 55 to both parent diabetics. There was a significant increase in IMT in T2D patients belonging to both parent diabetics compared to patients belonging to non-diabetic parents (Table 2). The hsCRP levels showed significant increase in T2D patients belonging to both parent diabetics compared to patients belonged to non-diabetic parents. There were no significant differences in other parameters (Table 2).

As shown in Table 3, the IMT increased significantly with increasing of age in T2D patients, as compared to healthy controls. The IMT values of T2D patients and controls were 0.78 ± 0.05 vs 0.67 ± 0.03 mm in 31-40 yrs, 0.86 ± 0.003 vs 0.75 ± 0.03 mm in 41-50 yrs and 0.92 ± 0.02 vs 0.76 ± 0.03 mm in age group 51-60 yrs, respectively.

Table 1—Physiological and biochemical parameters in diabetic patients, healthy controls, children of diabetic patients and healthy controls

[Values expressed as mean \pm SD]

Variables	Healthy controls (n = 200)	Diabetic patients (n = 200)	Children of healthy controls (n = 25)	Children of diabetic patients (n = 25)
Age (Yrs)	48.83 \pm 7.22	49.21 \pm 6.99	26.92 \pm 2.63	26.16 \pm 3.18
Body mass index (kg/m ²)	23.60 \pm 1.91	24.60 \pm 2.34	22.19 \pm 1.80	23.66 \pm 4.33
Systolic BP (mmHg)	122.82 \pm 5.60	128.95 \pm 5.46	118.88 \pm 2.09	117.36 \pm 6.99
Diastolic BP (mmHg)	81.72 \pm 4.33	87.07 \pm 4.94	79.2 \pm 2.24	81.20 \pm 8.16
Fasting plasma glucose (mg/dl)	91.23 \pm 8.52	160.04 \pm 34.38*	75.28 \pm 5.44	79.40 \pm 9.63
HbA1c (%)	5.51 \pm 0.18	7.55 \pm 0.68*	5.19 \pm 0.13	5.30 \pm 0.23
Serum total cholesterol (mg/dl)	172.25 \pm 10.61	214.44 \pm 22.66*	159.76 \pm 6.83	164.28 \pm 15.94
Serum triglycerides (mg/dl)	137.96 \pm 20.10	175.96 \pm 15.69*	117.16 \pm 11.22	122.76 \pm 8.33
Serum HDL-cholesterol (mg/dl)	38.55 \pm 2.32	30.21 \pm 2.35*	35.24 \pm 2.37	34.44 \pm 1.26
Serum LDL-cholesterol (mg/dl)	102.23 \pm 8.88	149.13 \pm 21.67*	92.56 \pm 6.34	93.40 \pm 6.42
ApolipoproteinB (mg/dl)	90.88 \pm 13.53	135.00 \pm 15.27*	81.72 \pm 9.00	82.16 \pm 5.66
hsCRP (mg/L)	2.28 \pm 0.36	3.66 \pm 0.58*	1.98 \pm 0.19	2.01 \pm 0.19
IMT (mm)	0.74 \pm 0.04	0.87 \pm 0.06*	0.60 \pm 0.03	0.61 \pm 0.02
Serum nitrite (μ mol/L)	43.88 \pm 2.57	34.06 \pm 4.18*	ND	ND

* $p<0.0001$ vs control; ND, Not done

Table 2—Physiological and biochemical parameters in diabetic patients with family history

Variables	[Values expressed as mean \pm SD]		
	Patients of non-diabetic parents (n = 64)	Patients of single parent diabetic (n = 81)	Patients of both the parent diabetic (n = 55)
Age (Yrs)	47.00 \pm 0.11	49.25 \pm 6.88	51.75 \pm 6.05
Body mass index (kg/m ²)	24.73 \pm 0.04	24.70 \pm 2.20	24.29 \pm 2.87
Systolic BP (mmHg)	124.34 \pm 0.11	125.80 \pm 4.79	124.29 \pm 9.08
Diastolic BP (mmHg)	83.22 \pm 0.09	83.78 \pm 3.87	84.11 \pm 8.22
Fasting plasma glucose (mg/dl)	162.58 \pm 0.45	160.65 \pm 39.37	156.18 \pm 32.60
HbA1c (%)	7.04 \pm 0.01	7.16 \pm 0.72	7.11 \pm 0.66
Serum total cholesterol (mg/dl)	208.42 \pm 0.33	217.40 \pm 22.29	217.07 \pm 24.00
Serum triglycerides (mg/dl)	171.47 \pm 0.27	178.38 \pm 15.24	177.60 \pm 13.01
Serum HDL-cholesterol (mg/dl)	30.59 \pm 0.04	30.26 \pm 2.28	29.69 \pm 2.33
Serum LDL-cholesterol (mg/dl)	144.04 \pm 0.32	151.52 \pm 21.20	151.55 \pm 23.31
Serum VLDL (mg/dl)	34.29 \pm 0.05	35.68 \pm 3.05	35.52 \pm 2.60
ApolipoproteinB (mg/dl)	131.56 \pm 0.28	135.74 \pm 14.23	137.89 \pm 12.23
hsCRP (mg/L)	3.35 \pm 0.01	3.75 \pm 0.57	3.81 \pm 0.61
IMT (mm)	0.84 \pm 0.01	0.87 \pm 0.05	0.92 \pm 0.04*

*P<0.05 vs patients of non-diabetic parents

Table 3—Age-wise distribution of IMT in type 2 diabetic patients and healthy controls

Age group (Yrs)	[Values expressed as mean \pm SD]	
	Healthy control (mm)	Diabetic patients (mm)
31-40	0.67 \pm 0.03	0.78 \pm 0.05*
41-50	0.75 \pm 0.03	0.86 \pm 0.03*
51-60	0.76 \pm 0.03	0.92 \pm 0.02*

*p<0.0001 vs control

Discussion

Cardiovascular complications are the leading cause of death among diabetic patients. Individuals with pre-diabetes, undiagnosed T2D and long-lasting T2D are at high risk of all complications of macrovascular disease, coronary heart disease (CHD), stroke and peripheral vascular disease. More than 70% of patients with T2D die of cardiovascular causes²³. The rising prevalence of T2D increases the burden of CVD. Measurement of IMT of common carotid artery is an excellent non-invasive technique to assess early cardiovascular risk and is also used to examine the stages of atherosclerosis²⁴.

In our study significant increase was observed in IMT in T2D patients, as compared with non-diabetic healthy controls. Also, IMT increased with the increase in the age of T2D patients, however, no significant increase was observed in healthy subjects. Earlier, in one of the study, it has been demonstrated that age, duration of diabetes, smoking and

hypertension may not result in increased IMT in diabetic patients²⁵. However, in another study, the age and HbA1c have been reported to be the risk factors for IMT in type 1 DM, but not in type 2 DM²⁶. Our study also showed significantly increased levels of serum total cholesterol, triglycerides, LDL-cholesterol, ApoB and hsCRP in T2D patients than healthy controls. An early study has also shown that hyperlipidemia and T2D are related for increasing IMT²⁷.

In our study, the IMT value of T2D patients increased according to their family history, i.e., non-diabetic parent, and single and both diabetic parents. But, their children in the age group 21-30 yrs, who were non-diabetic, did not show significant increase in IMT values than the children of healthy controls. The heritability of IMT studied in several different populations has shown that one-fifth to one-half of IMT variation is influenced by genetic factors²⁸⁻³⁰. In another study, IMT value is reported to be a good prognostic indicator for heart disease and stroke in diabetic patients³¹. Endothelial dysfunction is detectable in young normotensive first degree relatives of subjects with T2D patients³². As endothelial dysfunction represents an early disturbance in the development of atherosclerotic lesions, slightly elevated fasting and/or post-prandial glucose concentrations in offspring of T2D patients can cause a loss of endothelial function, leading to an increase in IMT.

Assessment of IMT is important for evaluating the atherosclerotic risk in people with diabetes and can further be used to facilitate better use of various treatment strategies in people with diabetes. Few studies have shown that along with hyperglycemia, other metabolic factors associated with diabetes that are known to increase cardiovascular risk, including obesity, insulin resistance, hypertension, hyperlipidemia and increased inflammatory state may contribute to progression of IMT in subjects with diabetes³³⁻³⁶.

In the present study, the ApoB level was increased in T2D patients than healthy controls. Earlier, similar results have been obtained in a cross sectional study carried out on consecutive patients of T2D³⁷. A positive association of plasma ApoB with T2D has been reported and shown to be a better predictor of risk as compared to LDL or HDL-cholesterol³⁸.

In the present study, the hsCRP levels were increased in T2D patients than the healthy controls. The elevated hsCRP is shown to be associated with the increased risk of future cardiovascular risk³⁹ and the presence of diabetes might aggravate the risk⁴⁰⁻⁴³. The association of hsCRP with T2D is independent of obesity, markers of hyperglycemia, dyslipidemia has been reported in the North Indian population⁴⁴.

Evidence supports endothelial dysfunction as a key early event in the pathogenesis of atherosclerosis⁴⁵. NO is a potent vasodilator and signaling molecule produced by many cells, including the vascular endothelium⁴⁶. A reduction in bioavailability of NO is found in atherosclerotic vessels before vascular structural changes occur⁴⁷. Increased expression and activity of arginase which metabolizes the NO substrate L-arginine might result in reduced production of NO, and thereby leading to endothelial dysfunction in T2D. Further, inhibition of arginase by N(ω)-hydroxy-nor-l-arginine has been reported to improve the endothelial function in patients of DM and CVD⁴⁸. In our study, increase in IMT in T2D patients might be due to decreased serum nitrite levels in T2D patients, which was in agreement with the early study⁴⁹. In another study, significantly low NO levels and significantly high plasma glucose and HbA1c levels have been reported in diabetic normotensive and diabetic hypertensive patients, as compared to controls⁵⁰.

In conclusion, the present study demonstrated that IMT was significantly higher in the T2D patients and increased with age and family history. The increased

levels of lipids, hsCRP, IMT and decreased nitrite levels might contribute to the risk of endothelial dysfunction in patients with T2D. However, further studies are warranted with other biomarkers of endothelial dysfunction in T2D patients with increased sample size.

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