STUDY OF LIPOPROTEIN PATTERNS AS AN ATHEROGENIC FACTOR IN T2DM PATIENTS AND THEIR FDRS

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Background: The present study was carried out to determine the lipoprotein patterns as an atherogenic factor in T2DM (Type 2 Diabetes Mellitus) patients and their FDRS (First Degree Relatives). In various previous studies it has been reported that hyperinsulinemia and hyperlipidemia frequently coexist in T2DM patients which indicate that the diabetic state it self is associated with atherogenic lipid disorders. **Method:** The present study included 26 Type 2DM (T2DM) patients and 21 apparently healthy First Degree Relative (FDRs) of T2DM patients. Twenty three age matched control not related to diabetics were also included in the study. **Results:** The BMI in male patients and FDRs were significantly higher as compared to controls but no significant difference was seen among the BMI of female FDRs. Fasting plasma glucose (FPG) levels of T2DM patients were significantly higher but no significant difference was observed among the FDRs and controls. FPG did not show any significant correlation with the BMI in diabetic patients, FDRs and controls. The lipid profile of patients showed no significant difference, except the mean LDL-Chol of female patients was significantly higher as compared to female controls. HDL-Chol of males FDRs was significantly lower as compared to male controls. LDL-Chol of female FDRs was significantly lower than the female controls. No significant difference was observed in the total cholesterol and the triglycerides level of the patients of T2DM and FDRs but those cases in which pre-beta band appear upon lipoprotein electrophoresis had significantly higher triglyceride levels as compared to those patients in which pre-beta band did not appear. **Conclusion:** Lipoproteins of the T2DM patients and FDRs group were found to be highly disturbed as compared to the control group and they show a trend of developing atherogenic states in future.

Key Words: Type 2 Diabetes Mellitus (T2DM), First Degree Relatives (FDRs), Body Mass Index (BMI), Lipoproteins, Fasting Plasma Glucose (FPG), Coronary Artery Disease

INTRODUCTION

It is anticipated that by the year 2025 the world will have over three hundred million diabetics and the majority will be Asians.[1] Insulin resistance is more common in overweight individuals and is associated with increased risk for T2DM and cardiovascular disease.[2] It was observed that the T2DM patients and their FDRs have insulin resistance and dyslipidemia.[3] The cause of lipid alteration among T2DM patients is differential insulin distribution which leads to increase in VLDL and triglyceride production through hepatic hyperinsulinemia. This is combined with decrease in the activity of lipoprotein lipase in the endothelium of adipose tissue and skeletal muscle.[4] The cardiovascular disease is the leading cause of death in T2DM patients and FDRs of such patients are also at increased risk of developing overt T2DM and cardiovascular disease.[5]

Lipoproteins and Apolipoproteins

Four main lipoproteins are generally described according to density, i.e., very low, Intermediate, low and high density lipoproteins (VLDL, IDL, LDL and HDL,) respectively, whereas chylomicrons are the largest and least dense lipoproteins[6]. The major apolipoproteins include apo E, apo B, apo A-I, apo A-II, apo A-IV, apo C-I, apo C-II and apo C-III. Specific apolipoproteins function in the regulation of lipoprotein metabolism through their involvement in the transport and redistribution of lipids among various cells and tissues, through their role as cofactor for enzymes of lipid metabolism or through their maintenance of the structure of the lipoprotein particles.[7]

Hyperinsulinemia and hyperlipidemia frequently coexist in T2DM patients which indicate that the diabetic state itself is associated with atherogenic lipid disorders.[8] The dyslipidemic component of insulin resistance is an atherogenic lipoprotein phenotype, its component include small dense LDL particles with higher atherogenic risk.[9] Hepatic lipase (HL) is involved in the metabolism of several lipoproteins and may contribute to the atherogenic lipid profile in T2DM patients.[10] Lipoproteins Fractions except HDL are estimated through calculations based on total Cholesterol and Triglycerides determination. Error in the TG and Cholesterol estimations may result in a misleading picture of lipoproteins profile. The inaccuracy and tedium of lipoprotein electrophoresis by earlier techniques has discouraged clinicians and pathologists in some cases with normal lipoprotein profile by

estimation based on calculations, the use of this useful technique in selected cases and fractionization of lipoproteins by electrophoresis may help in dealing the dyslipidemic state. The characterization of triglycerides rich lipoproteins is important to detect abnormality of triglycerides metabolism.[11]

MATERIALS AND METHODS

Twenty two T2DM patients, 21 FDRs of T2DM patients and 23 normal controls were included in the study. Physical examination for height, weight, blood pressure and BMI was carried out.

a: Fasting Plasma Glucose (FPG), Total Cholesterol, Triglyceride and HDL were determined on Dade Behering Dimension AR, Clinical Chemistry Analyzer.

b: LDL–Chol was evaluated by the use of formula:[12]

$$LDL = T.\ Cholesterol - \left(\frac{TG}{5} + HDL\right)$$

c: Lipoprotein Electrophoresis

Electrophoresis was done in all serum samples by using lipoprotein electrophoresis kit Paragon P/N 655901. Later the strips were scanned on densitometer and the relative percentage of each lipoprotein zone was calculated & cases classified according to Fredrickson's method.

d: Statistical analysis was carried out using SPSS software for calculating mean, SD, students 't' test, coefficient of correlation 'r' of different variables.

RESULTS

The present study included 26 T2DM patients, 21 apparently healthy FDRs of T2DM patients and 23 age-matched normal controls (Table-1).

Table-1: Distribution of males and females in different groups. Figure in parentheses indicate total number of cases in each group.

Group	Male	Female
Patients (26)	13	13
FDRs (21)	10	11
Controls (23)	10	13

There was no significant difference in mean age of subjects and controls. The mean weight of the male T2DM patients and male FDRs were significantly ($p<0.02$ and $p<0.05$) higher than respective controls (Table-2).

The BMI of male T2DM patients and male FDRs were seen to be significantly ($p<0.02$ and $p<0.05$) higher as compared to male controls (Table-2) The mean systolic and diastolic BP of female patients were significantly ($p<0.01$ and $p<0.05$) higher as compared to the mean systolic and diastolic BP of female controls (Table-2). Mean fasting plasma glucose levels of T2DM patients was significantly ($p<0.001$) higher as compared to their respective controls (Table-3). The FDRs showed no significant rise in their fasting glucose levels, though the mean FPG levels were higher as compared to controls. The lipid profile of T2DM patients showed no significant difference in the triglycerides, total cholesterol and the HDL-Chol levels as compared to controls, though the patient levels were on higher side. Only the mean level of LDL-Chol of female T2DM patients was significantly ($p<0.02$) higher as compared to female controls (Table-3).

In case of FDRs the mean HDL-Chol of male FDRs was significantly ($p<0.05$) lower as compared to male controls (Table-3).

Table-2: Age, height, weight, BMI and blood pressure of T2DM patients, FDRs and controls. (Mean±SEM)

Group	Sex	Age (Years)	Height (Cm)	Weight (Kg)	BMI	Systolic BP (mm Hg)	Diastolic BP (mm Hg)
Patients	Male (13)	50.7±1.83	167.1±1.83	*74.8±2.85	*26.8±0.92	122.2±2.81	80±2.59
	Female (13)	51±2.38	156.9±1.75	69.6±3.44	28.3±1.28	+131.8±2.80	++86.9±1.65
FDRs	Male (10)	46.3±3.69	171.8±2.77	++78.3±5.26	++26.3±1.45	122±4.10	87±2.60
	Female (11)	45.45±3.05	157.1±2.37	69.2±5.23	27.9±1.96	*106.4±3.88	84.1±3.00
Controls	Male (10)	47.3±2.14	169.1±2.88	63±3.69	22.1±1.31	119±3.05	79.5±3.20
	Female (13)	49.31±2.08	157.9±1.73	60.3±3.31	24.45±1.66	118.5±2.62	79.62±2.50

*$p<0.02$, +$p<0.01$, and ++$p<0.05$ as compared to controls.

Table-3: Lipid profile and FPG of T2DM patients, FDRs and controls. (Mean±SEM)

Groups	Sex	Triglycerides (mg/dL)	Cholesterol (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	FPG (mg/dL)	HDL/LDL ratio
Patients	Male (13)	212.9±34.13	192.7±10.69	40±2.08	110.4±12.33	*158±11.37	0.42±0.06
	Female (13)	153±25.75	201.2±9.81	51.2±2.97	+120.15±7.24	*186.4±18.56	++0.45±0.04
FDRs	Male (10)	184.6±45.42	182.1±14.53	++37.3±2.32	95.81±13.31	84.72±2.78	0.39±0.03
	Female (11)	184.36±38.86	151±7.53	43.9±4.71	++67.5±8.56	103.9±9.70	0.81±0.18
Controls	Male (10)	159.7±19.60	171±14.33	43.6±1.89	94.5±14.00	87.8±3.59	0.57±0.09
	Female (13)	136.84±14.56	177.15±11.39	52.15±2.62	92.15±7.85	84.15±4.69	0.62±0.06

*$p<0.001$, +$p<0.02$, and ++$p<0.05$ as compared to controls.

When the serum lipoproteins pattern on electrophoresis in T2DM patients, FDRs and controls were compared, it was observed that 46% and 50% male patients and male FDRs, 31% and 36% of female patients and female FDRs respectively, showed alpha, beta and pre–beta band as compared to 20% of male and 23% of female controls (Table-4). Rest of the three groups showed only normal pattern with two bands (alpha and beta).

Table-4: Occurrence of pre-beta band in T2DM patients, FDRs and controls.

Groups	Occurrence of pre beta band		
	Male	Female	Total
Patients (26)	6 (46%)	4 (31%)	10 (39%)
FDRs (21)	5 (50%)	4 (36%)	9 (43%)
Controls (23)	2 (20%)	3 (23%)	5 (22%)

Fredrickson's Type IIa hyperlipo-proteinemia was seen in 3 T2DM patients, 2 FDRs and 1 control. Fredrickson's Type III hyperlipo-proteinemia was observed in 2 patients of T2DM and I FDR, while none of controls showed this pattern. Fredrickson's Type IV was observed to be present equally, i.e., in 6 T2DM patients and 6 FDRs, whereas only in one control group (Table-5).

Table-5: Distribution of T2DM patients, FDRs and controls on the basis of types of hyperlipoproteinemia.

	Normal Pattern	Fredrickson's type of Hyperlipoproteinemia		
	Normal	Type IIa	Type III	Type IV
Patients (26)	15	3	2	6
FDRs (21)	12	2	1	6
Controls (23)	21	1	-	1

DISCUSSION

The present study was carried out to determine the lipoprotein as an atherogenic factor in T2DM patients and their FDRs. In various previous studies it has been reported that hyperinsulinemia and hyperlipidemia frequently coexist in T2DM patients which indicate that the diabetic state it self is associated with atherogenic lipid disorders.[8–10] The present study included 26 T2DM patients, 21 apparently healthy FDRs of T2DM patients and 23 age matched normal controls. The mean weight of the male T2DM patients and male FDRs were significantly (p<0.02 and p<0.05) higher than respective controls. The BMI of male T2DM patients and male FDRs were seen to be significantly (p<0.02 and p<0.05) higher as compared to male controls. It has been reported that T2DM patients had markedly higher values of BMI as compared to healthy volunteers.[13, 14] Chang *et al* (2004)[15] reported that the BMI is the most important determinant of insulin resistance in non obese patients with T2DM Korea. A higher BMI in FDRs of T2DM patients was reported as compared to normal control, and the female FDRs of T2DM patients have 40% greater risk of developing DM as compared to female FDRs with normal BMI.[5,16,32] The mean systolic and diastolic BP of female patients were significantly (p<0.01 and p<0.05) higher as compared to the mean systolic and diastolic BP of female controls. Increased levels of blood pressure in T2DM patients has been reported earlier (2003).[17,18] The role of hypertension in increasing micro and macro-vascular complications has been confirmed in patients with T2DM.[19] Mean fasting plasma glucose levels of T2DM patients was significantly (p<0.001) higher as compared to their respective controls, earlier workers has also reported high FPG in T2DM patients.[20–23]

The lipid profile of T2DM patients showed no significant difference in the triglycerides, total cholesterol and the HDL-Chol levels as compared to controls, though the patient levels were on higher side. Only the mean level of LDL–Chol of female T2DM patients was significantly (p<0.02) higher as compared to female controls. The patients of T2DM with poor glycaemic control have shown increase levels of LDL-Chol as compared to normal controls.[24] It was observed that an improved glucose control is associated with lower lipid levels.[25] In case of FDRs the mean HDL-Chol of male FDRs was significantly (p<0.05) lower as compared to male controls. It has been reported by various workers that T2DM patients has elevated levels of total cholesterol, LDL-Chol, VLDL-Chol, hypertriglyceridemia and reduced levels of HDL-Chol.[23,26–30] Thus FDRs of T2DM patients also have higher risk for CAD as higher lipid levels and significant decrease in HDL-Chol has been reported.[31,32] When the serum lipoproteins pattern on electrophoresis in T2DM patients, FDRs and controls were compared. It was observed that 46% and 50% male patients and male FDRs, 31% and 36% of female patients and female FDRs respectively, showed alpha, beta and pre-beta band as compared to 20% of male and 23% of female controls.

Rest of the subjects in three groups showed only normal pattern with two bands (alpha and beta). Noble (1986)[33] has reported that the clear cut separation of lipoproteins by this method will facilitate the classification of hyperlipoproteinemia and improve quantitative estimates of lipoprotein distribution. Fredrickson's Type IIa hyperlipo-proteinemia was seen in 3 T2DM patients, 2 FDRs and 1 control. Earlier this type of pattern was observed in persons taking high cholesterol diet and also in some diseases like, nephrotic syndrome, dysglobulinemia and hypothyroidism.[34]

Fredrickson's Type III hyperlipoproteinemia was observed in 2 patients of T2DM and I FDR,

while none of controls showed this pattern. Fredrickson's Type IV was observed to be present equally, i.e., in 6 T2DM patients and 6 FDRs, whereas only in one control group. These findings are in agreement with the observations by various workers and presence of Fredrickson's Type IV, Type IIa and Type IIb dyslipidemia in diabetic patients has been documented earlier.[35,36] On the contrary Genest *et al* (1992)[37] reported that T2DM and obesity was not associated with Type II or Familial Hypercholesterolemia and a slender physique is typical in this case. However no comparable data was available on the type of dyslipidemia in FDRs of T2DM patients. Whereas Durruty *et al* (1998)[38] have reported a negative association between HDL-Chol and alpha lipoproteins in T2DM patients with coronary complications. In present study lipoproteins of the T2DM patients and FDRs were found to be highly disturbed as compared to control group.

Thus it appears that the FDRs of T2DM patients are also at higher risk for atherosclerosis and coronary artery disease (CAD). The degree of glycaemia and dyslipidemia related to micro vascular & macro vascular complications require special attention for correction.

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