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Original Research Article

Lipid Profile as a Marker of Cardiovascular Disease in Type 2 Diabetics with and without Nephropathy

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Abstract: Among the microvascular complications of type 2 diabetes mellitus, second most common complication is diabetic nephropathy (after retinopathy). Overt dyslipidemia may aggravate the condition, and it is also known to increase the risk of cardiovascular diseases (CVDs). The present study included 110 type 2 diabetic individuals each with and without nephropathy and 110 age and gender matched healthy non-diabetic individuals. The concentrations of FBG, HbA1c, TC, TGs, and high density lipoprotein cholesterol (HDL-C) were estimated from fasting blood samples. Significant increase was found in BMI, FBG, HbA1c, TC, TGs, LDL-C, VLDL-C, serum urea and creatinine in type 2 diabetic patients with as well as without nephropathy as compared to healthy individuals, while HDL-C was significantly decreased. The increased levels of urine creatinine and higher albumin/creatinine ratio were observed in type 2 diabetic nephropathy subjects as compared to healthy controls. Patients with proteinuria had significantly higher BMI, TC, TGs, LDL-C, VLDL-C, Serum urea, serum creatinine, urine creatinine, microalbumin, and albumin/creatinine ratio when compared to the patients with microalbuminuria. Hence, lipids and lipoproteins along with stage of nephropathy should be considered for prediction of CVDs in type 2 diabetic subjects with & without nephropathy.

Keywords: Cardiovascular diseases, diabetic nephropathy, total cholesterol (TC), triglycerides (TGs), type 2 diabetes mellitus (T2DM).

INTRODUCTION

From the rapid rise of diabetes incidence, the projected increase in the number of individuals is supposed to rise from 6.4% (285 million) in 2010 to 7.7% (439 million) in 2030 [1]. The important difficulty with this condition is its predisposition to suffer macro and/or microvascular complications as diabetes progresses, increasing the economic burden on the individuals and healthcare system as well [2].

One of the most serious life threatening and irreversible complication associated with diabetes mellitus is diabetic nephropathy [3, 4]. World Health Organization (WHO) projected, about 20-40% of both types of diabetes mellitus patients develop diabetic nephropathy within 20-30 years of the onset of diabetes [5]. It is characterized by continuous albuminuria, elevated blood pressure, declines glomerular filtration rate and high risk of CVDs [6].

The development and progression of diabetic nephropathy include various hyperglycemia-induced metabolic and hemodynamic derangements that involve increased formation of advanced glycation end products (AGEs), enhanced reactive oxygen species (ROS) generation and Protein Kinase C (PKC) activation, polyol pathway and Rennin Angiotensin System (RAS) [5, 7]. It represents the most common form of end-stage renal disease (ESRD) worldwide, accounting for increase in mortality rate in these patients [2, 8]. In developing countries like India, the high economic burden for treatment of ESRD, many of these patients are not getting optimal treatment [4].

According to estimates ever third individual with diabetes mellitus are affected and is related with substantial cardiovascular morbidity and mortality [7]. A number of risk factors have been identified from longitudinal and cross-sectional studies, in the development of diabetic nephropathy that includes race, genetic susceptibility, high blood pressure, hyperglycemia, hyperfiltration, smoking, male gender, age and dyslipidemia [5, 9]. The present study focused on lipid abnormalities in T2DM population with & without nephropathy and its comparison to normal healthy individuals.

MATERIAL AND METHODS

Present study comprised of, 110 T2DM patients each with and without nephropathy of either gender within the age limit of 35-60 years were included. Patients visiting Medicine Department, BVDU Medical College and Hospital, Pune were included in the study. T2DM patients, having cardiovascular, pulmonary diseases, with diabetic complications other than nephropathy, pregnant and lactating women and those on insulin therapy were excluded from the study. In addition to T2DM patients with and without nephropathy, age and gender matched 110 healthy non-diabetic individuals were also included in the study. The healthy individuals had no history of cardiovascular disorders or any major illness. The research protocol was accepted by Institutional Ethic's Committee of BVDU Medical College, Pune and informed written consent was obtained from every individual after complete explanation of the procedure.

The blood samples were collected by venepuncture after fasting of 12 hours. Plasma from the blood collected in fluoride vacutainer was used for estimation of fasting blood glucose (FBG) using commercially available kit (ERBA Diagnostics, Mannheim, Germany), and glycated hemoglobin (HbA1c) was estimated from samples collected in EDTA vacutainer by HPLC technique (D10, Bio-Rad). The serum was separated from plain vacutainer after centrifugation, and used for estimation of urea, creatinine, TC, TGs and high density lipoprotein cholesterol (HDL-C). Low Density Lipoprotein cholesterol (LDL-C) and Very Low Density Lipoprotein cholesterol (VLDL-C) were calculated by Friedwald's formula. Urine samples collected were used for estimation of urine creatinine and microalbumin level, and from these values the ratio of albumin to creatinine were calculated. All biochemical parameters were estimated by standard commercially available kits (ERBA Diagnostics, Mannheim, Germany).

Continuous variables were presented as mean ± standard error (SE) and differences in means between T2DM individuals with and without nephropathy and healthy individuals were compared using ANOVA. Further, the post hoc Tukey's test was used to evaluate the difference between any two groups. The difference in the means of biochemical parameters in T2DM patients with microalbuminuria and proteinuria were compared by student 't' test for independent samples. Difference in the means of groups was considered significant if $P \leq 0.05$.

RESULTS

In the present study, lipid profile, fasting glucose, HbA1C, blood urea, serum creatinine, urine creatinine & microalbumin were studied in T2DM patients with and without nephropathy and age, gender matched healthy controls. Each group had 110 individuals recruited for the study. Results are expressed as mean ± SE. The means were compared by ANOVA test and considered significant if $P \leq 0.05$.

There was no notable difference found in mean age of healthy individuals (48.36±0.69) and T2DM individuals with nephropathy (51.0±0.73) and without nephropathy (50.94±0.75). Mean body mass index (BMI) differed significantly between healthy controls (24.31±0.24) and T2DM patients with (27.78±0.25) and without nephropathy (26.96±0.25).

Table 1: Comparison of biochemical parameters between T2DM patients with and without nephropathy, and healthy controls

PARAMETERS	CONTROLS (n=110) Mean ± SE	T2DM Without Nephropathy (n=110) Mean ± SE	T2DM With Nephropathy (n=110) Mean ± SE
Fasting blood glucose (mg/dL)	97.62 ± 1.43	131.56 ± 1.52	185.76 ± 7.41
HbA1c (%)	5.35 ± 0.16	7.87 ± 0.14	8.86 ± 0.25
Blood urea (mg/dL)	23.27 ± 0.47	38.21 ± 0.77	74.5 ± 4.51
Serum creatinine (mg/dL)	1.12 ± 0.027	1.53 ± 0.042	2.96 ± 0.20
Urine creatinine (g/L)	0.91 ± 0.025	1.21 ± 0.027	5.2 ± 0.20
Urine microalbumin (mg/L)	15.70 ± 0.34	18.93 ± 0.35	300.48 ± 15.95
Albumin/Creatinine ratio (mg/g)	17.78 ± 0.30	15.82 ± 0.16	55.13 ± 1.70

Note: All parameters were found to be statistically significant ($p \leq 0.001$) for comparison between T2DM patients with & without nephropathy and healthy controls.

Table 2: Comparison of biochemical parameters between T2DM patients with and without nephropathy, and healthy controls

PARAMETERS	CONTROLS (n=110) Mean ± SE	T2DM Without Nephropathy (n=110) Mean ± SE	T2DM With Nephropathy (n=110) Mean ± SE
Total cholesterol (mg/dL)	171.97 ± 2.24	194.52 ± 2.57	204.1 ± 4.38
TGs (mg/dL)	136.12 ± 3.31	163.49 ± 2.36	192.8 ± 7.86
HDL-C (mg/dL)	48.07 ± 0.36	44.17 ± 0.57	39.15 ± 0.95
LDL-C (mg/dL)	96.67 ± 2.26	117.65 ± 2.44	126.03 ± 3.70
VLDL-C (mg/dL)	27.23 ± 0.66	32.69 ± 0.47	38.91 ± 1.57

Note: All parameters were found to be statistically significant ($p \leq 0.001$) for comparison between T2DM patients with & without nephropathy and healthy controls.

There was a statistically remarkable ($p \leq 0.05$) dissimilarity between the groups for the levels of FBG, HbA1c, TC, TGs, HDL-C, LDL-C, VLDL-C, blood urea, serum creatinine, urine creatinine, microalbumin and albumin/creatinine ratio (Table 1 and 2).

Further, Tukey's post hoc test revealed statistically significant increase in levels of BMI, FBG, HbA1c, TC, TGs, LDL-C, VLDL-C, blood urea and serum creatinine, and decrease in HDL-C in T2DM patients without nephropathy and healthy controls. All these parameters in addition to urine creatinine, urine microalbumin and albumin/creatinine ratio were observed to be significantly altered between T2DM patients with nephropathy and healthy controls. T2DM patients with nephropathy exhibited significant alteration in the levels of FBG, HbA1c, TGs, HDL-C, VLDL-C, blood urea, serum creatinine, urine creatinine, urine microalbumin and albumin/creatinine ratio as compared to those without nephropathy.

Table 3: Comparison of biochemical parameters in DN patients with microalbuminuria and proteinuria

PARAMETERS	MICROALBUMINURIA (n=55) Mean ± SE	PROTEINURIA (n=55) Mean ± SE
BMI (Kg/m^2)	26.4 ± 0.24	29.2 ± 0.35**
Fasting Blood Glucose (mg/dL)	184.1 ± 11.27	186.46 ± 9.73
HbA1c (%)	8.97 ± 0.34	8.74 ± 0.38
Blood urea (mg/dL)	49.12 ± 2.55	99.89 ± 7.21***
Serum creatinine (mg/dL)	1.84 ± 0.08	4.12 ± 0.32***
Urine creatinine (g/L)	3.42 ± 0.12	7.08 ± 0.16***
Urine microalbumin (mg/L)	142.25 ± 4.87	458.72 ± 8.75***
Albumin/Creatinine ratio (mg/g)	44.15 ± 2.1525	66.11 ± 1.6029***

**$p \leq 0.01$, ***$p \leq 0.001$

Table 4: Comparison of Lipid profiles in DN patients with microalbuminuria and proteinuria

PARAMETERS	MICROALBUMINURIA (n=55) Mean ± SE	PROTEINURIA (n=55) Mean ± SE
Total cholesterol (mg/dL)	181.70 ± 4.47	226.43 ± 6.24***
TGs (mg/dL)	162.73 ± 7.92	222.80 ± 12.40**
HDL-C (mg/dL)	40.91 ± 1.12	37.39 ± 1.51
LDL-C (mg/dL)	108.29 ± 4.47	143.77 ± 6.72***
VLDL-C (mg/dL)	32.55 ± 1.58	45.29 ± 2.48**

**$p \leq 0.01$, ***$p \leq 0.001$

Patients with proteinuria had significantly higher BMI, TC, TGs, LDL-C, VLDL-C, blood urea, serum creatinine, urine creatinine, microalbumin and albumin/creatinine ratio when compared to the patients with microalbuminuria. The results are depicted in Table 3 and 4.

DISCUSSION

The prevalence of diabetes is increasing globally with an anticipated two fold rise of diabetic population from 171 million in 2000 to 366 million in 2030. The considerable increase will occur in Middle Eastern countries, Sub Saharan Africa and India. These diabetic individuals are susceptible to increased risk of both micro and/or macro-vascular complications. The mortality by cardiovascular diseases is about 50% in diabetic population. Atherosclerosis, might be initiated during the diagnosis of diabetes, and deformities in lipid metabolism can be a cause of increased cardiovascular complications in such patients [10].

India has a huge burden of diabetes and consequently more vulnerability to complications like kidney disease. Diabetes leading to ESRD and death

due to cardiovascular events is a devastating medical calamity of wide ranging magnitude. Hyperinsulinemia aggravates hypertension through several pathways, including lipoprotein abnormality. There is evidence that lipid reduction might preserve GFR, decrease proteinuria and thereby reducing the rate of major vascular events [6, 11].

Patients with diabetes mellitus are found to be higher arterial stiffness, which may evolve due to increased protein glycation, resulting in progression of arteriosclerosis [12]. Lipoprotein abnormalities are more common and contributes to coronary heart diseases in diabetic nephropathy patients [13].

It is suggested by number of observational studies, lipids may be involved in the development and advancement of glomerular injury, which are consistent with the results of the present study. Kathore *et al* [13]. found higher TC, TGs, VLDL-C, LDL-C levels, LDL-C/HDL-C and TGs/HDL-C ratios and decreased HDL-C level in diabetic individuals with & without nephropathy in comparison of controls. Suchitra *et al* [14] reported atherogenic dyslipidemia with elevation in Lp(a), TC, TGs, VLDL-C, LDL-C, non-HDL-C, ratios of the lipids and decreased HDL levels in both T2DM patients with & without nephropathy than controls. Significantly higher TG/HDL-C, TC/HDL-C were also observed in diabetic kidney patients as compared to T2DM.

In the present study lipids and lipoprotein levels were estimated. The levels of FBG, HbA1c, TGs, HDL-C, VLDL-C, blood urea and serum creatinine levels were altered in between all the groups studied. The BMI, TC and LDL-C were altered in T2DM patients with and without nephropathy than healthy controls. The urine creatinine, microalbumin and albumin/creatinine ratio were differed significantly between the T2DM patients with nephropathy than healthy controls and T2DM patients without nephropathy. Further the patients divided on the basis of urine protein excretion, patients having proteinuria had higher BMI, TC, TGs, LDL-C, VLDL-C, blood urea, serum creatinine, urine creatinine, microalbumin, and albumin/creatinine ratio than microalbuminuric patients.

Our earlier study demonstrated significant increase in FBG, HbA1c, TC, TGs, LDL, VLDL-C levels, TC/HDL-C, TG/HDL-C, LDL-C/HDL-C ratios, non HDL-C & cholesterol retention fraction levels, and significant decrease in HDL-C level in T2DM patients than controls [15]. Study of Khadke *et al*. [16] reported the significant altered levels TC, LDL-C and HDL-C but TGs level was not affected among T2DM patients and controls. Samatha *et al*. [17] found similar results i.e. significant increase in fasting blood sugar, glycated hemoglobin, blood urea nitrogen, microalbumin, TC and decreased HDL-C values in cases with diabetes as compared to those of the controls. Significantly elevated levels of serum TC, TGs and LDL-C, and significantly lower levels of serum HDL have also been reported in patients with diabetes [18].

The results consistent with present study were encountered by Al-Jameil *et al.* [6], TC, TGs, LDL-C, VLDL-C values were significantly increased in diabetics than normoalbuminuria, microalbuminuria and overt proteinuria and were found to be positively correlated with albumin to creatinine ratio. Sigdel *et al*. [19] noted significantly decreased HDL-C and marginally higher fasting blood sugar, TGs, TC and VLDL-C in patients with microalbuminuria than in normoalbuminuric individuals. Mean TC and non-HDL-C were higher in microalbuminuric than in normoalbuminuric patients [20].

Glomerular and tubulointerstitial injury caused by elevated lipoproteins and lipids in diabetes mellitus contribute to the progression of diabetic nephropathy. However, treatment of dyslipidemia can reduce albumin excretion. Early hemodynamic changes of glomerular hyperperfusion and hyperfiltration are followed by leakage of albumin from the glomerular capillaries and structural changes such as glomerular basement membrane thickening, glomerular hypertrophy, glomerulosclerosis, mesangial cell expansion, and podocyte injury [6].

Increasing diabetic prevalence will inevitably result in increasing mortality due to CVD, diabetic nephropathy as well as other complications of diabetes mellitus [6]. Dyslipidemia plays a vital part in progression of kidney abnormalities in diabetic patients and ends with CVD complications. Diabetes mellitus leads to dyslipidemia and this aggravated dyslipidemia is seen with diabetic patients with nephropathy. Lipid alterations related to diabetes mellitus and diabetic nephropathy include increased levels of TGs, VLDL-C, IDL cholesterol, LDL-C and low level of HDL-C [4].

CONCLUSION

The lipids and lipoprotein levels may increase the risk of CVD in T2DM patients with & without nephropathy. Therefore lipids, lipoproteins along with the stages of nephropathy should be considered for prediction of CVDs in T2DM patients with and without nephropathy. It will help in management of dyslipidemia, thereby decreasing the risk of CVDs in these patients.

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