OPEN ACCESS
Scholars International Journal of Biochemistry
Abbreviated Key Title: Sch Int J Biochem
ISSN 2616-8650 (Print) |ISSN 2617-3476 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: http://saudijournals.com/sijb/

Original Research Article

Progression of Proteinuria in Type 2 Diabetes Mellitus Patients: Role of Lipid Profile Estimation

Chirag K Pandya[1], Keyur H Madhu[1*], Tejas J Shah[2], Subhankar Kayal[3]
[1]Resident Doctor, Department of Biochemistry, Govt. Medical College, Bhavnagar, Gujarat, India
[2]Associate Professor, Department of Biochemistry, SBKS Medical Institute and Research Centre, Piparia, Vadodara, Gujarat, India
[3]Tutor, Department of Biochemistry, Rampurhat Govt. Medical College & Hospital, West Bengal, India

***Corresponding author:** Dr. Keyur H Madhu
| **Received:** 09.05.2019 | **Accepted:** 16.05.2019 | **Published:** 30.05.2019
DOI:10.21276/sijb.2019.2.5.4

Abstract

Background and Objectives: Diabetic nephropathy (DN), a common microvascular complication in diabetic patients, is characterized by gradually increasing urinary albumin excretion (UAE), and affects 20-40% of Type 2 Diabetes mellitus (DM) patients in India. The defective insulin action in the metabolism of lipoproteins leads to lipid abnormalities as well as glycation of lipoproteins cause abnormalities in the lipoprotein metabolism in Type 2 DM patients. Dyslipidemia causes glomerular injury which will lead to progression of proteinuria. ***Methods:*** 100 Type 2 Diabetic patients with not less than 10 years duration of diabetes were selected and divided in three groups; Normoalbuminuric (n=42), Microalbuminuric (n=41) and Macroalbuminuric (n=17). Lipid profile, HbA1c, S. Creatinine and Urinary Albumin Excretion (UAE) were measured and data analysis was performed. ***Result:*** Total Cholesterol (TC), Triglyceride (TG) and LDL-C levels were not significantly associated with increase in proteinuria. HDL-C levels and TG/HDL ratio were significantly associated with different stages of proteinuria. ***Conclusion:*** HDL–C levels and TG/HDL ratio can be considered an excellent tool and approach for estimating risk of development of overt proteinuria among Type 2 diabetes patients.

Keywords: Microalbuminuria, Type 2 DM, Diabetic Nephropathy, TG/HDL ratio.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder of various etiologies andit is characterized by chronic hyperglycemia with disturbances of carbohydrate, protein and fat metabolism due to deficiency of insulin secretion, insulin action or both. According to its etiology DM is classified as Type 1 DM and Type 2 DM which is associated with the development of the specific microvascular as well as macrovascular complications.

Diabetic nephropathy remains an important common microvascular complication of diabetes which leads to End-stage renal disease (ESRD), a devastating condition to the individual and society of massive financial and social consequences. Diabetic nephropathy is a chronic condition, characterized by gradually increasing urinary albumin excretion (UAE) from microalbuminuria to overt proteinuria and decreasing glomerular filtration rate (GFR). The risk of other chronic complications like cardiovascular disease (CVD) increases dramatically as nephropathy progresses [1].

Moorhead *et al*., [2] in their study noted an association between hyperlipidemia and glomerular capillary injury. They suspected that the continual filtration of lipids and lipoproteins promote progression of chronic renal injury [2]. Several other consequent observational studies supported the role of increased serum lipids in the development of albuminuria and in the progression of glomerulosclerosis [3, 4]. Gall *et al*., [5] in their study showed a significant association between total serum cholesterol and increased urinary albumin excretion. Klein *et al*., [6] reported that patients with type 1 diabetes with higher total serum cholesterol and lower high-density lipoprotein cholesterol (HDL-C) developed a higher incidence of renal insufficiency. The lipid profiles of individuals with DN have been characterized to have higher plasma concentrations of very low density lipoprotein cholesterol (VLDL-C), low-density lipoprotein cholesterol (LDL-C), intermediate-density lipoprotein cholesterol, and triglycerides but lower levels of HDL-C [7]. An increase in hepatic lipase activity and a decreased post heparin plasma lipoprotein lipase (LPL) ratio have also been documented [8, 9]. Furthermore in patients with

long duration of diabetes, glycation of lipoproteins causes abnormalities in the lipoprotein metabolism, such as glycated LDL can be taken up by macrophages that form foam cells as it is not cleared by the physiological LDL receptor (LDL-R) pathway and thus increases risk of atherosclerosis [10].

The defective insulin action in the metabolism of lipoproteins leads to lipid abnormalities in DN which become more accentuated with worsening renal function and urinary albumin excretion [2, 11, 12]. This causes enhanced lipolysis with ensuing increase in free fatty acids and VLDL-C synthesis, a defect in LPL activity leading to the increased life span of chylomicrons and VLDL-C in circulation, an increased transfer of cholesterol esters resulting in triglyceride-rich LDL-C, and lastly the rise of plasma triglycerides and the decreased ratio of LPL to hepatic lipase causing the accelerated breakdown of HDL-C [13].

Under this backdrop and literature review, association of lipids with the development and progress of Diabetic nephropathy in Type 2 DM should require to be studied. Hence, our key objective was to study involvement of serum lipids and lipoproteins in the development of overt proteinuria in diabetic nephropathy patients.

MATERIAL & METHOD

A prospective case control study was conducted for the period of one year between July 2017 to June 2018. This study was reviewed and approved by Human Ethics Committee of Government Medical College, Bhavnagar, Gujarat, India. Total 100 adult patients with not less than 10 years duration of Type 2 Diabetes Mellitus, being treated and followed up in the Diabetic clinic at Sir Takhtsinhji General Hospital, Bhavnagar were included. Patients suffering from diseases causing proteinuria other than diabetic nephropathy like drug induced nephrotoxic damage, having proteinuria before the onset of diabetes, obstructive renal disease, renal stone disease, acute urinary tract infection, congestive cardiac failure and pregnancy were excluded.

Medical history of each participant was taken & anthropometric parameters like weight (Kg) and height (m) were measured, and Body Mass Index was calculated by weight /height squared (Kg/m^2). Venous blood sampling was done for biochemical determinations in Plain and EDTA vacutainer in the atleast 8 hours fasting state and early morning urine sample was collected for analysis of urinary albumin excretion (UAE). Serum was separated by centrifugation for the analysis of following biochemical parameters. Plasma Glucose (Glucose oxidase method, CV%: 4.8), S. Creatinine (Modified Jaffe's method, CV%: 6.8), S. Total Cholesterol (Cholesterol oxidase method, CV%: 4.7), S. Triglycerides (Enzymatic method, CV%: 3.6), S. HDL-C (Immunoinhibition method, CV%: 8.5), S. LDL-C (Enzyme selective protection method, CV%: 4.5), HbA1c (Immunoturbidimetric method, CV%: 4.9) The urine samples are tested for UAE by immunoturbidimetric assay (CV%: 4.9).

According to urinary albumin excretion (UAE) the patients were divided into 3 groups. Group A (Normoalbuminuric group): Patients with UAE < 30 mg/day (n=42,), Group B (Microalbuminuric group): Patients with UAE between 30-300 mg/day (n=41), Group C (Macroalbuminuric group): Patients with UAE > 300 mg/day (n=17).

Data analysis of this study was done in GraphPad InStat version 3.0 by using Kruskal – Wallis (nonparametric ANOVA) test & Dunn's multiple comparison test. Interpretation of the result was done according to p-value where $p < 0.001$ – highly significant, $p < 0.05$ – significant and $p \geq 0.05$ – not significant.

RESULT

The mean age for Group A was 58 ± 5.1 years, Group B was 57.1 ± 6.1 years and Group C was 61.3 ± 4.4 years. Female (n=53) participants were more than male (n=47). Mean of BMI for Group A was 26.4 ± 2.1, Group B was 27.3 ± 2.0 and Group C was 26.7 ± 2.3. Mean of HbA1c levels for Group A was 7.7± 0.9, Group B was 7.7± 1.1 and Group C was 7.7 ± 1.3. Mean of S. Creatinine levels for Group A was 0.9 ± 0.1, Group B was 0.9 ± 0.1 and Group C was 1.4 ± 0.3.

Mean of S. Total Cholesterol (TC) levels for Group A was 188 ± 35, Group B was 191 ± 41 and Group C was 201 ± 45. Mean of S. Triglyceride (TG) levels for Group A was 173 ± 52, Group B was 174 ± 62 and Group C was 202 ± 78. Mean of S. HDL-C levels for Group A was 49 ± 9.0, Group B was 47 ± 6.1 and Group C was 40 ± 5.8. Mean of S. LDL-C levels for Group A was 113 ± 28, Group B was 117 ± 31 and Group C was 130 ± 29. Mean of S. VLDL-C levels for Group A was 34.7 ± 10.4, Group B was 35.0 ± 12.5 and Group C was 40.4 ± 15.4. Mean of TG/HDL ratio for Group A was 3.7 ± 1.8, Group B was 3.8 ± 1.7 and Group C was 5.2 ± 2.3 (Table-1).

Table 1: Comparison of Study Group A, B & C

Parameters	Group A- Normoalbuminuric Group (n=42)	Group B- Microalbuminuric Group (n=41)	Group C- Macroalbuminuric Group (n=17)	Significance
	Mean ±SD	Mean±SD	Mean±SD	
Age (years)	58 ± 5.1	57.1 ± 6.1	61.3 ± 4.4	-
Sex Males, n(%) Females, n(%)	 21 (50%) 21 (50%)	 20 (48.7%) 21 (51.3%)	 07 (41.2%) 10 (58.8%)	-
BMI (Kg/m^2)	26.4 ± 2.1	27.3 ± 2.0	26.7 ± 2.3	p= 0.0974$^{\#}$
HbA1C (%)	7.7 ± 0.9	7.7 ± 1.1	7.8 ± 1.3	p= 0.8550$^{\#}$
S. Creatinine (mg/dl)	0.9 ± 0.1	0.9 ± 0.1	1.4 ± 0.3	p <0.0001**
S. Total Cholesterol (mg/dl)	188 ± 35	191 ± 41	201 ± 45	p= 0.6578$^{\#}$
S. Triglyceride (TG) (mg/dl)	173 ± 52	174 ± 62	202 ± 78	p= 0.4228$^{\#}$
S. HDL – C (mg/dl)	49 ± 9.0	47 ± 6.1	40 ± 5.8	p < 0.0001**
S. LDL – C (mg/dl)	113 ± 28	117 ± 31	130 ± 29	p= 0.0701$^{\#}$
S. VLDL – C (mg/dl)	34.7 ± 10.4	35.0 ± 12.5	40.4 ± 15.4	p= 0.4350$^{\#}$
TG/HDL ratio	3.7± 1.8	3.8± 1.7	5.2 ± 2.3	p= 0.0059*

*p < 0.05 – significant, **p < 0.001 – highly significant, $^{\#}$p ≥ 0.05 – not significant

Table-2: Correlation of HDL-C levels among three study Groups

	Mean Rank Difference	Statistical Significance
Group A vs. Group B	3.644	p > 0.05$^{\#}$
Group A vs. Group C	35.889	p < 0.001**
Group B vs. Group C	32.245	p < 0.001**

*p < 0.05 – significant, **p < 0.001 – highly significant, $^{\#}$p ≥ 0.05 – not significant

Table 3: Correlation of TG/HDL ratio among three study Groups

	Mean Rank Difference	Statistical Significance
Group A vs. Group B	2.884	p > 0.05$^{\#}$
Group A vs. Group C	-23.062	p < 0.05*
Group B vs. Group C	-25.945	p < 0.01*

*p < 0.05 – significant, **p < 0.001 – highly significant, $^{\#}$p ≥ 0.05 – not significant

DISCUSSION

Diabetes is becoming a potential epidemic in India with more than 62 million diabetic individuals currently diagnosed with the disease and it will increase up to 79.4 million cases by 2030 [14]. As diabetic prevalence is increasing it will without doubt result in increasing proportions of death from complications of DM like CVD and diabetic nephropathy, these complications are associated with dyslipidemia [13].

In our study, patients of all study groups were closely matched for age & sex. Slightly elevated levels of HbA1c were seen in macroalbuminuric group (Group C) but there was no statistically significant difference between all the groups (Table-1). HbA1c is gold standard for measurement of glycemic control over prolong period of time and It is also considered as a predictor of diabetic complications.

In our study, serum creatinine was found to be increased in macroalbuminuric group (Group C) and it was statistically highly significant. It suggests that high levels of S. Creatinine associated with renal injury in overt proteinuria patients which is in accordance with Prasad P *et al.*, [15].

Most diabetic patients have dyslipidemia and it is recently been proposed as a major risk factorfor progression of DN [13]. In our study we found that S. Total Cholesterol levels, S. triglyceride levels, S. LDL-

C levels and S. VLDL levels were elevated in macroalbuminuric patients than microalbuminuric patients than normoalbuminuric patients, but the rise in levels were not statistically significant. Our findings are partially in accordance to Shoji *et al*., [16] and differs from findings of Noura Al-Jameil *et al*., [17].

S. HDL- C levels were significantly decreased with increase in proteinuria. (Table-1) Correlation of HDL-C levels among three study Groups revealed that in between normoalbuminuric and macroalbuminuric patients and also in between microalbuminuric and macroalbuminuric patients there was a significant decrease in mean values of HDL- C levels but the decrease in the mean values of HDL-C levels in between normoalbuminuric and microalbuminuric patients was not statistically significant. Our findings are consistent with Shoji *et al.*, [16] and Ejuoghanran OSO *et al*., [18], while differ with Noura Al-Jameil *et al*., [17] It is well established that decreased HDL-C levels increases risk of cardiovascular complication because of its role in cellular cholesterol efflux.

Previously conducted prospective studies demonstrated that, increased triglyceride-to-HDL ratio (TG/HDL ratio) is independently associated with the progression of microalbuminuria in type 2 DM [19]. TG/HDL ratio was significantly increased with increase in proteinuria (Table-1). Correlation of TG/HDL ratio among three study Groups revealed that in between normoalbuminuric and macroalbuminuric patients and also in between microalbuminuric and macroalbuminuric patients there was a significant decrease in mean values of TG/HDL ratio but the decrease in the mean values of TG/HDL ratio in between normoalbuminuric and microalbuminuric patients was not statistically significant. Our findings are consistent with Retnakaran R *et al.*, [20].

CONCLUSION

From the present study it is evident that levels of S. Total Cholesterol, S. Triglyceride, S. LDL-C and S. VLDL- C are not significantly associated with progression of proteinuria in Type 2 DM patients. While S. HDL–C levels and TG/HDL ratio are significantly associated with progression of proteinuria in Type 2 DM patients and they can be considered as an excellent tool and approach for estimating risk of development of overt proteinuria among Type 2 DM patients. Therefore, it is suggested that determination of TG/HDL ratio, Life style modifications and pharmacological interventions for dyslipidemia should be implemented to prevent or delay conversion of microalbuminuria to overt proteinuria in the Type 2 DM patients.

REFERENCES

1. Holt, R. I., Cockram, C., Flyvbjerg, A., & Goldstein, B. J. (Eds.). (2017). *Textbook of diabetes*. John Wiley & Sons.
2. Moorhead, J. F., El-Nahas, M., Chan, M. K., & Varghese, Z. (1982). Lipid nephrotoxicity in chronic progressive glomerular and tubulo-interstitial disease. *The Lancet*, *320*(8311), 1309-1311.
3. Krolewski, A. S., Warram, J. H., & Christlieb, A. R. (1994). Hypercholesterolemia--a determinant of renal function loss and deaths in IDDM patients with nephropathy. *Kidney International Supplement*, (45), S125–S131.
4. Ravid, M., Neumann, L., & Lishner, M. (1995). Plasma lipids and the progression of nephropathy in diabetes mellitus type II: effect of ACE inhibitors. *Kidney international*, *47*(3), 907-910.
5. Gall, M. A., Hougaard, P., Borch-Johnsen, K., & Parving, H. H. (1997). Risk factors for development of incipient and overt diabetic nephropathy in patients with non-insulin dependent diabetes mellitus: prospective, observational study. *Bmj*, *314*(7083), 783-788.
6. Klein, R., Klein, B. E., Moss, S. E., Cruickshanks, K. J., & Brazy, P. C. (1999). The 10-year incidence of renal insufficiency in people with type 1 diabetes. *Diabetes Care*, *22*(5), 743-751.
7. Bonnet F, Cooper ME: Potential influence of lipids in diabetic nephropathy. insights from experimental data and clinical studies. Diabetes Metab 2000; 26:254–264.
8. Kahri, J., Groop, P. H., Elliott, T., Viberti, G., & Taskinen, M. R. (1994). Plasma cholesteryl ester transfer protein and its relationship to plasma lipoproteins and apolipoprotein AI-containing lipoproteins in IDDM patients with microalbuminuria and clinical nephropathy. *Diabetes care*, *17*(5), 412-419.
9. Groop, P. H., Elliott, T., Friedman, R., Viberti, G., Ekstrand, A., Franssila, A. K., & Taskinen, M. R. (1996). Multiple lipoprotein abnormalities in type I diabetic patients with renal disease. *Diabetes*, *45*(7), 974-979.
10. Verges, B. (2005). New insight into the pathophysiology of lipid abnormalities in type 2 diabetes. *Diabetes & metabolism*, *31*(5), 429-439.
11. Jensen, T., Stender, S., & Deckert, T. (1988). Abnormalities in plasmas concentrations of lipoproteins and fibrinogen in type 1 (insulin-dependent) diabetic patients with increased urinary albumin excretion. *Diabetologia*, *31*(3), 142-145.
12. Jerums, G., Allen, T. J., Tsalamandris, C., Akdeniz, A., Sinha, A., Gilbert, R., & Cooper, M. E. (1993). Relationship of progressively increasing albuminuria to apoprotein (a) and blood pressure in type 2 (non-insulin-dependent) and type 1 (insulin-dependent) diabetic patients. *Diabetologia*, *36*(10), 1037-1044.

13. Rosario, R. F., & Prabhakar, S. (2006). Lipids and diabetic nephropathy. *Current diabetes reports*, *6*(6), 455-462.
14. Wild, S., Roglic, G., Green, A., Sicree, R., & King, H. (2004). Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes care*, *27*(5), 1047-1053.
15. Prasad, P., Tiwari, A. K., Kumar, K. P., Ammini, A. C., Gupta, A., Gupta, R., ... & Tiwari, S. C. (2006). Chronic renal insufficiency among Asian Indians with type 2 diabetes: I. Role of RAAS gene polymorphisms. *BMC medical genetics*, *7*(1), 42-50.
16. Shoji, T., Emoto, M., Kawagishi, T., Kimoto, E., Yamada, A., Tabata, T., ... & Nishizawa, Y. (2001). Atherogenic lipoprotein changes in diabetic nephropathy. *Atherosclerosis*, *156*(2), 425-433.
17. Al-Jameil, N., Khan, F. A., Arjumand, S., Khan, M. F., & Tabassum, H. (2014). Dyslipidemia and its correlation with type 2 diabetic patients at different stages of proteinuria.
18. Ejuoghanran, O. S. O., Chukwu, O. E., & Christopher, S. L. (2011). The effect of diabetic nephropathy on the lipid profile of diabetics in Southern Nigeria. *J Med Sci*, *11*, 198-202.
19. Smulders, Y. M., Rakic, M., Stehouwer, C. D., Weijers, R. N., Slaats, E. H., & Silberbusch, J. (1997). Determinants of progression of microalbuminuria in patients with NIDDM: a prospective study. *Diabetes care*, *20*(6), 999-1005.
20. Retnakaran, R., Cull, C. A., Thorne, K. I., Adler, A. I., & Holman, R. R. (2006). Risk factors for renal dysfunction in type 2 diabetes: UK Prospective Diabetes Study 74. *Diabetes*, *55*(6), 1832-1839.