ORIGINAL ARTICLE

Lipoprotein(a) and LDL-Cholesterol Status in type 2 Diabetes Mellitus with Microvascular Complications

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ABSTRACT

The present study was conducted to investigate lipid profile in T2DM patients with microvascular complications such as nephropathy, retinopathy and neuropathy. Case-control observational study in Medical Research Unit (MRU), of Medical and Health Wealfare Trust (MHWT), Uttara, Dhaka, Bangladesh from October 2013 to December 2014; A total of 150 T2DM (Group-B) patients (male: 90, female: 60, age range: 25-65 years) with 30 patients in each sub-group, i.e. without complications (B1), with nephropathy (B2), with retinopathy (B3), with neuropathy (B4) and with multiple complications (B5) and 30 normal controls (male: 18, female: 12, age range: 28-60 years)(Group-A) were included in the study. The lipid profile i.e. triglyceride (TG,) total cholesterol (TC), LDL-C, HDL-C and Lp(a) were quantitatively measured by standard clinical laboratory methods. The findings were compared statistically among patients and controls. Serum lipids i.e. TG, TC, LDL-C and Lp(a) were elevated and HDL-C was decreased in patients (Group -B) compared to controls (Group-A) significantly [Group-A vs Group-B: TG (mg/dl) - 93.7$\pm$18.9, 184.4$\pm$36.5; TC (mg/dl) - 141.9$\pm$25.5, 237.7$\pm$69.5; LDL-C (mg/dl) - 85.8$\pm$22.1, 165.1$\pm$26.3; HDL-C (mg/dl) -47.4$\pm$17.4, 35.5$\pm$6.6; Lp(a) (mg/l) - 29.1$\pm$14.2, 73.5$\pm$23.4] ($P < 0.001$). Among microvascular complications, T2DM-patients with nephropathy (Group-B2) had the highest elevated levels of TG, TC, LDL-C and Lp(a) and maximally decreased level of HDL-C ($P < 0.001$); Our findings suggest that reduction of all cholesterol-bearing lipoproteins that contain apoprotein B would be important in T2DM with microvascular complications. Possibly Lp(a) reduction and induction of HDL-C are most relevant in this regard.

Short Title: Lp(a) & LDL-C status in T2DM patients

Key Words: T2DM, Lipoprotein (a), Nephropathy, Retinopathy, Neuropathy

Introduction

Diabetes mellitus (DM), particularly type 2 diabetes mellitus (T2DM), is a major public health problem in both developed and developing countries and the world is witnessing a diabetes pandemic. It is expected that the estimated number of patients with DM 300 million by 2025.[1,2] The resource burden of the pandemic will fall primarily on the developing countries, as DM is a chronic disease with devastating atherosclerotic complications including microangiopathy such as diabetes retinopathy, nephropathy and neuropathy and macroangiopathy such as coronary artery disease (CAD), cardiovascular disease (CVD) and diabetic foot.[2,3]

Among the microangiopathies, diabetic retinopathy is probably the most characteristic, easily identifiable and treatable complication of DM and it remains an important cause for visual loss in the developing world. Since T2DM remains undiagnosed for several years, a significant number of people, even in developed countries, already have retinopathy by the time their diabetes is diagnosed.[3,4] Secondly, diabetic nephropathy is the most common cause of end-stage renal disease in many countries. Microalbuminuria is believed to be a strong predictor of diabetic nephropathy. It is recommended that all diabetic patients should have an annual measurement of albumin in the urine.[3,5] Thus, it has become an important function of any diabetic clinic to assess the eye and kidney statuses of T2DM patients.[1,6,7] Diabetic neuropathy, another long-term complication of diabetes, is a relatively common complication affecting approximately 30% of diabetic patients. The nerves most commonly affected are the 3rd and 6th cranial nerves resulting in diplopia and femoral and sciatic nerves.[8,9] Central nervous system (CNS) is affected in long term diabetes, although the clinical impact of diabetes is mainly manifested in the peripheral nervous system (PNS).[10,11]

Current evidence supports the role of nearly all lipoproteins, particularly low density lipoprotein-cholesterol (LDL-C) and high density lipoprotein-cholesterol (HDL-C) in the pathogenesis of atherosclerosis.[12,13] The recent report of the National Cholesterol Education Programme (NCEP) mainly focused on the modification of LDL-C to < 70 mg/dl in high-risk patients. The NCEP report acknnowledges the limitations of pharmacotherapy in achieving the optional serum LDL-C reduction goal(< 70 mg/dl), as it varies from 31-45% with different statins.[13,14] Although the principal focus is on serum LDL-C currently, more rational approach would be to reduce the concentrations of all cholesterol-bearing lipoproteins that contain apoprotein B. The lipoprotein (a) [Lp(a)] is the most important and relevant one in this regard.[14] However, it appears that the report of NCEP did not give due consideration about the role of Lp(a) in atherosclerosis.

Based on the similarity of Lp(a) to both LDL and plasminogen, it has been hypothesized that the function of this unique lipoprotein may represent a link between the fields of atherosclerosis and thrombosis.[15,16] Although Lp(a) has been shown to accumulate in atherosclerotic lesions, its contribution to the development of atheromas is unclear. Only limited studies have been reported on serum levels of Lp(a) in some populations including Indian subcontinent.[16,17,18] Serum Lp(a) levels are reported to be elevated in T2DM and it is an independent risk factor for CAD in DM, particularly T2DM patients.[18,19] One study on serum Lp(a) level in patients with cerebrovascular disease was reported earlier from Bangladesh.[19] Recently, another study showing elevation of serum Lp(a) level in patients with T2DM was reported from Bangladesh.[20]

Literature review indicated that no studies comparing the role of Lp(a) with LDL-C and other lipoproteins have been reported in T2DM patients with microvascular complications such as retinopathy, nephropathy and neuropathy from Bangladesh. The present case-control prospective observational study was therefore undertaken to investigate the blood lipid profile, i.e. triglyceride (TG), total cholesterol (TC), LDL-C, HDL-C, and Lp(a) in T_2DM patients with microvascular complications, i.e. nephropathy, retinopathy, neuropathy and multiple complications and compared with healthy normal controls.

Patients & Methods

This is a case-control observational study and T2DM cases and among diabetics with complications such as nephropathy, retinopathy, neuropathy and multiple conducted at Medical

Research Unit (MRU), The Medical & Health welfare Trust (MHWT), Medical College for Women and Hospital (MCW&H) , Uttara, Dhaka, Bangladesh. Ethical permission was taken from the concerned Departments & Authorities. All the study subjects were informed and explained about the nature of the study and included only after having their consent. The total number of study subjects were 180 classified into groups, i.e. Group A normal healthy controls (n= 30, male=18, female=12, age range= 28-60 years, mean age±SD = 42.5±10.5 years) and Group B T2DM (total) (n=150, male=90, female=60, age range =25-65 years, mean age± SD=45.5±11.5 years). Then Group B patients (n=150) were further classified into 5 (five) categories i.e. B1 - T2DM without complications (n=30); B2 - T2DM with nephropathy (n=30); B3 - T2DM with retinopathy (n=30); B4 - T2DM with neuropathy (n=30) and B5 - T2DM with mixed complications (n=30). Patients with confirmed history of diabetes without and with nephropathy, retinopathy, neuropathy and mixed complications were included in the study. The kidney, eye and peripheral neuropathy with diabetic foot status of the patients were determined either prospectively or from the medical records of the diabetic clinic at MCW&H, Uttara, Dhaka, Bangladesh.[5,6,7,21,22,23,24] Regarding neuropathy there were polyneuropathy and mononuropathy. Symptoms included paraesthesia in the feet and in the hands, pain in the lower limbs, burning sensation in the soles of the feet, cutaneous hyperasthesia and abnormal gait, muscle weakness and wasting developed in advanced cases.[10,11] Age and gender matched healthy volunteers with no known disease were also included as normal healthy controls. Patients suffering from heart diseases, taking lipid lowering drugs therapy, acute and chronic systemic illnesses, thyroid disorders, billiary diseases and other renal diseases were excluded from the study.

After obtaining consent, clinical findings were recorded as per proforma designed for each patient and 6-10 ml fasting blood samples were collected from each subject with full aseptic precaution and taking care to avoid haemolysis. Blood was allowed to clot and then centrifuged at 2000 rpm, separated serum was aliquoted in eppendroff tube appropriately labeled and then stored frozen until analyzed for serum lipid profile, i.e. TG, TC, HDL-C, LDL-C and Lp(a). All quantitative estimations in serum were made by standard clinical laboratory methods such as estimation of serum LP(a) by immunonephelometric method, TC by enzymatic end point CHOD-PAP method, TG by enzymatic colorimetric GPO-PAP method, HDL-C by enzymatic colorimetric phosphotungstate/magnesium method using standard diagnostics kits from internationally reputed companies and LDL-C calculated by Friedwald formula.[25] The results were analysed statistically by Student's t- test and ANOVA using SPSS program in computer.[26]

Results

Table 1 shows the serum levels of lipid parameters and their statistical analyses in normal controls (Group A) and in cases/patients (Group B). Serum TG, TC, LDL-C and Lp(a) levels were elevated and HDL-C level was reduced in patients (Group B) significantly ($p<0.001$). In Group B, Lp(a) concentration was 73.5+23.4 mg/dl which was significantly higher in comparison to that of 29.11±14.2 mg/dl in Group A ($p<0.001$).

Table-2 shows the comparison by ANOVA of mean serum concentrations of every lipid parameter, i.e. TG, TC, LDL-C, HDL-C and Lp(a) among Group A, Group B_1, Group B_2, Group B_3, Group B_4 and Group B_5. All lipid parameters in all patient groups were significantly higher than controls individually. Among micro vascular complications, Group B_2 patients had highest elevated levels of TG, TC,

LDL, Lp(a) and maximally decreased level of HDL-C ($P<0.001$).

Table- 3 shows the comparison of the lipid parameters between sub-groups by Student's t test. TG concentration among different groups were significantly higher compared to control (Groups A vs B1, B2, B3, B4 & B5) ($p<0.001$). However, comparison among patient groups showed that TG level was not significantly raised between B1 vs B3 ($p=0.208$) and B1 vs B5 ($p=0.75$). The comparison between sub-groups for TC revealed that TC concentration among different groups were significantly higher compared to controls (Groups A vs B_1, B_2, B_3, B_4 & B_5) ($p<0.001$). Among patient groups, TC concentrations were not significantly raised between B_1 vs B_3 ($p=0.535$), B_1 vs B_4 ($p=0.274$) and B_1 vs B_5 ($p=0.213$). Also, LDL-C concentration among different groups were significantly higher compared to controls (Groups A vs B_1, B_2, B_3, B_4 & B_5) ($p<0.001$). Among patient groups, LDL-C concentrations were not significantly raised between B_1 vs B_3 ($p=0.368$), B_3 vs B_4 ($p=0.05_3$) and B_4 vs B_5 ($p=0.061$).

HDL-C levels were similar between A vs B_4 ($P=0.076$), B_1 vs B_3 ($p=0.226$) and B_1 vs B_5 ($p=0.086$). Interestingly, HDL-C levels among different patient groups were significantly lower compared to controls (Groups A vs B_1, B_2, B_3, B_4 & B_5) ($p<0.001$). Importantly, Lp(a) concentrations among different patient groups were significantly higher compared to controls (Groups A vs B_1, B_2, B_3, B_4 & B_5) ($p<0.001$). However, among patient groups, Lp(a) concentrations were not significantly raised between B_1 vs B_3 ($p=0.749$), B_2 vs B_5 ($p=0.379$) and B_3 vs B_5 ($p=0.054$) .

Table-I: Comparison between Group A (Normal Controls) and Group B (T_2DM patients) for serum lipid parameters by Student's t-test

Lipid parameters (mg/dl)	Groups Group A (n=30) (Mean±SD)	Group B (n=150) (Mean±SD)	Student's t - test* t	df	p
TG	93.7±18.9	184.4±36.5	13.25	178	<0.001*
TC	141.9±25.5	237.7±69.5	7.44	178	<0.001*
LDL-C	85.8±22.1	165.1±26.3	15.45	178	<0.001*
HDL-C	47.4±17.4	35.5±6.6	6.43	178	<0.001*
Lp (a)	29.1±14.2	73.5±23.4	10.03	178	<0.001*

* $p<0.05$: Significant; $p>0.05$: Not significant

Table-II: Comparison among groups for each lipid parameter by ANOVA

Laboratory parameters (mg/dl)	Groups A (n=30) Mean±SD	B_1 (n=30) Mean±SD	B_2 (n=30) Mean±SD	B_3 (n=30) Mean±SD	B_4 (n=30) Mean±SD	B_5 (n=30) Mean±SD	F-ratio	df	P
TG	93.7±18.9	180.4±41.3	220.8±23.9	170.0±17.5	153.6±24.9	197.3±29.7	77.12	5	<0.001*
TC	141.9±25.5	220.1±69.4	314.6±37.4	209.5±61.9	202.9±49.2	241.4±61.6	33.84	5	<0.001*
LDL-C	85.8±22.1	147.0±30.5	188.0±14.0	152.7±16.1	163.3±24.4	174.6±20.7	78.80	5	<0.001*
HDL-C	47.4±17.4	36.7±6.8	30.6±5.2	35.1±3.5	41.2±7.5	34.1±4.3	13.83	5	<0.001*
Lp (a)	29.1±14.2	69.5±23.7	87.3±20.4	71.4±21.1	56.9±17.3	82.4±22.4	32.81	5	<0.001*

* $p<0.05$ significant; $p>0.05$: Not significant

Group A: Normal controls; Group B_1 : T_2DM without complications;

Group B_2 : T_2DM with nephropathy; Group B_3 : T_2DM with retinopathy;

Group B_4 : T_2DM with neuropathy); Group B_5: T_2DM with mixed complications

Table-III: Comparison by Student's t-test between groups for lipid parameters

Group compared	TG (mg/dl)			TC (mg/dl)			LDL-C (mg/dl)			HDL-C (mg/dl)			Lp (a) (mg/L)		
	t	df	p	t	df	P	T	df	p	t	df	p	t	df	P
A vs B_1	-10. 46	58	<0.001 *	-5.80	58	<0.001 *	-8.90	58	<0.001 *	3.15	58	0.003 *	-8.02	58	<0.001 *
A vs B_2	-22.8	58	<0.001 *	-20.90	58	<0.001 *	-21.37	58	<0.001 *	5.08	58	<0.001 *	-12.85	58	<0.001 *
A vs B_3	-16.21	58	<0.001 *	-5.53	58	<0.001 *	-13.39	58	<0.001 *	3.81	58	<0.001 *	-9 .11	58	<0.001 *
A vs B_4	-10.48	58	<0.001 *	-6.04	58	<0.001 *	-12.87	58	<0.001 *	1.81	58	0.076 ns	-6.81	58	<0.001 *
A vs B_5	-16.13	58	<0.001 *	-8.18	58	<0.001 *	-16.06	58	<0.001 *	4.07	58	<0.001 *	-11.03	58	<0.001 *
B_1 vs B_2	-4.63	58	<0.001 *	-6.57	58	<0.001 *	-6.70	58	<0.001 *	3.89	58	<0.001 *	-3.12	58	0.003 *
B_1 vs B_3	1.27	58	0.208 ns	0.62	58	0.535 ns	-.91	58	0.368 ns	1.12	58	0.266 ns	-.322	58	0.749 ns
B_1 vs B_4	3.05	58	0.003 *	1.10	58	0.274 ns	-2.28	58	0.026 *	2.44-	58	0.018 *	2.35	58	0.022 *
B_1 vs B_5	-1.81	58	0.075 ns	-1.26	58	0.213 ns	-4.11	58	<0.001 *	1.75	58	0.086 ns	-2.17	58	0.034 *
B_2 vs B_3	9.39	58	<0.001 *	7.86	58	<0.001 *	9.07	58	<0.001 *	3.96-	58	<0.001 *	2.97	58	0.004 *
B_2 vs B_4	10.65	58	<0.001	9.89	58	<0.001	4.81	58	<0.001	6.36-	58	<0.001	6.22	58	<0.001
B_2 vs B_5	3.38	58	0.001 *	5.57	58	<0.001 *	2.94	58	0.005 *	2.85-	58	0.006 *	0.89	58	0.379 ns
B_3 vs B_4	2.95	58	0.005 *	0.45	58	0.653 ns	-1.98	58	0.053 *	4.03-	58	<0.001 *	2.90	58	0.005 *
B_3 vs B_5	-4.33	58	<0.001 *	-2.00	58	0.05 *	-4.58	58	<0.001 *	0.99	58	0.326 ns	-1.96	58	0.054 *
B_4 vs B_5	-6.17	58	<0.001 *	-2.67	58	0.01 *	-1.94	58	0.06 ns	4.48	58	<0.001 *	-4.94	58	<0.001 *

* $p < 0.05$ significant; $p > 0.05$: Not significant(ns)
Group A: Normal controls; Group B_1: T2DM without complications;
Group B_2: T2DM with nephropathy; Group B_3: T2DM with retinopathy;
Group B_4 : T2DM with neuropathy; Group B_5: T2DM with mixed complications

Discussion

This case-control prospective study is the first report from Bangladesh on serum lipid profile which includes lipoprotein (a) as well in T2DM patients with and without complications. The present study shows that serum levels of TG, TC and LDL-C, are elevated while HDL-C is reduced in T_2DM patients. Our findings that serum Lp(a) level is significantly elevated in T2DM without and also with microvascular complications are consistent with some reports from other countries.[7]

Lp(a) has become a focus of research interest owing to the results of case- control and prospective studies linking its elevated blood level with CAD. Serum Lp(a) level was reported to be elevated in T2DM and an independent risk factor for CAD and also for CAD in T2DM.[19,20,24] Elevated blood levels of Lp(a) (>30 mg/dl) were reported to confer an increased risk of CAD and, because of this association, the measurement of plasma Lp(a) is requested increasingly as part of CAD risk assessment.[18,20,24,25] Our study shows that Lp(a) level in the blood is elevated associated with development and progression of retinopathy, nephropathy and neuropathy in T2DM patients and possibly, a correlation exits between the severity of diabetic microvascular complications. Recently, a number of studies have been reported on the role of Lp(a) in T2DM patients with microvascular complications such as retinopathy, nephropathy and neuropathy.[27-34] It was reported that increased serum Lp(a) levels correlated with higher degree of retinopathy.[27,28] However, Hashem et al reported higher serum TG and LDL-C and lower HDL-C levels in Bangladeshi T2DM patients with retinopathy.[29] Although

these observations are similar to our findings, they did not investigate the serum Lp(a) levels to compare with our results as stated in Tables-I,II,III.

Abd-Allha et al, Song et al, Dwivedi et al and Chang etal demonstrated that Lp(a) is an independent risk factor for the progression of nephropathy in T2DM patients with overt proteinuria.[9,10] Lakhotia et al reported results from India similar to our findings of significantly higher Lp(a) levels in T2DM patients with nephropathy.[32] Gazzaruso et al reported that higher Lp(a) level was associated with vascular diabetic foot, while lower Lp(a) level appeared to be associated with delayed wound healing in T2DM patients with neuropathic foot ulceration.[33] Although our patients with neuropathy (Group B4) had the lowest value among the different patient groups, Group B4 also had significantly raised Lp(a) level compared to controls (Group A) (Table-II). This was in contrary to the report that no association between Lp(a) level and diabetic neuropathy or retinopathy were observed.[34] Another important aspect is that baseline Lp(a) levels were not measured in cases and controls in many follow-up studies with cholesterol lowering therapy. However, some studies showed that cholestyramine treatment was not effective in lowering Lp(a). Statins alone or in combination reduce the plasma levels of Lp(a), although the probable beneficial effects of lowering serum Lp(a) levels in CAD risk reduction by statins have not been considered which remained to be evaluated and answered.[13,14] In recent overviews on the management of primary hyperlipidemia by statins, blood baseline Lp(a) levels and its reduction were not mentioned and considered in the discussion.[16,17,19] Even the updated NCEP, USA report published in July 2004 discussed and debated LDL-C only and consideration for Lp(a) level was not suggested in the NCEP report.[14]

Lp(a) contains a low-density lipoprotein (LDL)-like moiety, in which the apolipoprotein B-100 component is covalently linked to the unique glycoprotein apolipoprotein(a) [Apo(a)]. Apo(a) is composed of repeated loop-shaped units called kringles, the sequences of which are highly similar to a kringle motif present in the fibrinolytic proenzyme plasminogen.[15,17] Because of sequence homology with plasminogen, Lp(a) may compete with plasminogen for binding to fibrin and impair fibrinolysis. High levels of Lp(a) in blood may therefore represent a potential source of antifibrinolytic activity.[15,17] In addition to this antifibrinolytic activity, high concentration of Lp(a) also suppresses the activity of transforming growth factor-beta (TGF-β) which has the potential to inhibit the proliferation of endothelial cells and smooth muscle cells. This probably causes increased proliferation of the vascular endothelial cells and smooth muscle cells resulting in the progression of atherosclerosis.[10,17] So, treatment of hypercholesterolemia with cholestyramine/ statines may reduce but can not abolish progression of atherogenesis and hence risk of long-term complications in T2DM. These clearly indicate that in the studies with cholesterol lowering drugs such as cholestyramine/statines, blood Lp(a) levels should be followed up as well. Lp(a) measurement may have a significant role to play in the prediction and management of patients relevant to atherosclerosis including long-term complications such as CAD and stroke in DM patients.[33,34]

In conclusion, our findings of elevated serum Lp(a) levels in T2DM patients without and with microvascular complications were consistent with some reports in the literature and possibly have very important implications in the development of microvascular complications in T2DM patients. The fact that plasma Lp(a) levels are largely genetically determined and vary widely among different ethnic groups adds scientific interest to the ongoing research on this enigmatic particle/molecule. Further studies are required involving larger number of T2DM patients correlating blood Lp(a) level with those of other lipids particularly LDL-C and HDL-C and the severity of long-term complications such as retinopathy, nephropathy, neuropathy and multiple complications.

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