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The relationship between lipoprotein (a) levels and microvascular complications in patients with type 2 *Diabetes mellitus*

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ABSTRACT

Lipoprotein (a) is known to have thrombotic and atherogenic features. Considering thrombotic and atherogenic features of Lp (a), the present study was planned to investigate the significance of lipoprotein (a) as a risk factor for the development of microvascular and macrovascular complications of diabetes.

A total of 71 subjects were included in the study. In order to assess nephropathy, 24-hour urine samples were collected and microalbuminuria levels were measured. Ophthalmologists evaluated presence of retinopathy by eye-ground examination. Neuropathy was detected via anamnesis, physical examination, and electroneuromyography .

Thirty-six percent of the patients had nephropathy, 26% had retinopathy and 22% had neuropathy. There were no significant difference between Lp (a) levels and vascular complications ($P > 0.05$).

There were no statistically significant differences between Lp (a) and micro- or macrovascular complications in patients with type 2 diabetes.

Key words: nephropathy, retinopathy, neuropathy, lipoprotein (a).

INTRODUCTION

Diabetes mellitus is the most commonly encountered endocrine disease in the world. *Diabetes mellitus* is a syndrome characterized by chronic hyperglycemia, impairment in carbohydrate, protein, and lipid metabolisms, capillary membrane alternations and accelerated development of atherosclerosis as a result of complete or partial deficiency of endogenous insulin or its peripheral ineffectiveness.

Chronic complications of diabetes are results of its various effects. Blood glucose levels, alterations in lipid metabolism, impaired thrombocyte functioning, and various other factors are known to have roles in the development of these complications. Chronic complications of diabetes can be divided into two: microvascular and macrovascular. Although the development of macrovascular complications is obviously related to the duration of diabetes; such a relationship is not significant for microvascular complications[1]. Microvascular complications of diabetes are nephropathy, neuropathy, and retinopathy; and macrovascular complications are cerebrovascular diseases, ischemic heart disease, and peripheral arterial disease[2].

Although lipoprotein (a) [Lp (a)] is known for 35 years, sufficient evidence about its role in coronary artery disease as an independent risk

factor was revealed in the recent years [3]. In-vitro studies showed that Lp (a) plays a direct role in atherogenesis via cholesterol uptake, or an indirect role via inhibiting fibrinolysis[4]. Although the function of Lp (a) is not clearly understood, we know that it has thrombotic and atherogenic features.

On the basis of this information, we aimed to investigate the significance of Lp (a) as a risk factor for the development of microvascular and macrovascular complications of diabetes, besides the other risk factors; including hyperglycemia, hypertension, impaired lipid profile, obesity and smoking.

MATERIAL AND METHODS

A total of 71 patients (49 women and 22 men), who referred to diabetes outpatients clinic of Taksim Training and Research Hospital between April-July 2002 and were diagnosed as type 2 diabetes mellitus according to American Diabetes Association (ADA, 1997) criteria, were included in the study[5]. Patients' age, duration of diabetes, and diagnosis of arterial hypertension were questioned and recorded. Body mass index (BMI) was calculated as weight/square of height (kg/m^2).

Presence of retinopathy was assessed by ophthalmologists via eye-ground examination.

Twenty-four hour urine samples were collected from patients for microalbuminuria. Patients were instructed to avoid stress and tiring exercises before collection of their urine samples. 1 mL sample from collected urine was analyzed via immunoturbidimetry method, using Roche/Hitachi modular auto analyzer with Tina-quant albumin kit. Patients were classified according to excreted albumin levels in their urines:

- Normoalbuminuria: < 30mg/day
- Microalbuminuria: 30-300 mg/day
- Macroalbuminuria: >300 mg/day

Presence of neuropathy was detected with anamnesis, physical examination, and electroneuromyography (ENMG). Distal latency and conduction velocity of motor and sensory action potentials in both upper and lower extremities were measured using Medelec-MS92 device. F-wave responses were also evaluated.

Lp (a) was analyzed in Roche/Hitachi modular auto analyzer using immunoturbidimetric method

Total cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol, uric acid and FBG were evaluated in Roche/Hitachi 912 auto analyzer with enzymatic colorimetric method. Ratio of total cholesterol/HDL-cholesterol was calculated based on total cholesterol and HDL cholesterol levels.

Urine and blood creatinine levels were measured simultaneously and creatinine clearance was calculated with the formula below:

Creatinine clearance = Urine volume X urinary creatinine/1440 X serum creatinine

Statistical analysis

Statistical analysis of the data was performed using SPSS (Statistical Package for Social Sciences) software for Windows (version 10.0). During analysis of quantitative data, in addition to descriptive statistics (mean, standard deviation), Student-t test and Mann Whitney U test were used for normally and non-normally distributed data, respectively. Comparison of the qualitative data was performed using Chi-square test. Pearson correlation analysis was used to detect associations between parameters. Risk factors that affected hypertension, retinopathy, microalbuminuria and neuropathy were ranked by logistic regression analysis. Results were evaluated within the range of % 95 confidence interval with a significance level of $P < 0.05$.

RESULTS

This study was conducted on a total of 71 patients (49 women and 22 men), who referred to diabetes outpatient clinics of Taksim Training and Research Hospital between April-July 2002 and were diagnosed as type 2 *diabetes mellitus* according to American Diabetes Association criteria (ADA, 1997)[5]. Descriptive data of the patients are shown in Table 1.

Thirty-six percent of the patients had microalbuminuria. Mean age of the patients with microalbuminuria was higher than others (t=2.32; *P*=0.023). No statistically significant difference was

Table 1: Descriptive statistics for all patient data

	Minimum	Maximum	Average ± SD
Mean age (years)	40.00	70.00	56.69 ± 9.19
BMI (kg/m^2)	19.00	40.00	29.33 ± 2.86
HbA1c (%)	5.70	15.80	8.14 ± 3.68
Creatinine clearance (ml/min)	33.10	227.30	112.66 ± 34.49
Total cholesterol (mg/dl)	112.00	344.00	210.63 ± 47.24
Triglyceride (mg/dl)	54.00	584.00	165.46 ± 101.8
HDL- cholesterol (mg/dl)	27.00	77.00	44.57 ±11.85
LDL- cholesterol (mg/dl)	59.00	252.00	131.63 ± 37.6
Total Chol/HDL cholesterol	2.30	8.70	4.91 ±1.38
FBG (mg/dl)	85.00	476.00	179.43 ±3.68
BUN (mg/dl)	8.00	34.00	16.32 ± 4.93
Creatinine (mg/dl)	0.43	1.14	0.75 ± 0.16
Uric acid (mg/dl)	1.80	8.30	4.89 ± 1.41
Lp (a) (mg/dl)	6.00	145.10	38.35 ± 31.40

* P < 0.05 significant, ** P < 0.01 highly significant.

Table 2: Incidences of microangiopathic and macroangiopathic complications in the patients

Complications	N	%
Neuropathy	16	22.0
Hypertension	40	56.0
Microalbuminuria	26	36.0
Ischemic heart disease	19	26.0
Retinopathy	19	26.0
Total number of patients	71	100

observed between Lp (a) levels, and rates or means of the other variables (*P*>0.05) (Table 3).

Retinopathy was observed in 26% of the patients. In patients with retinopathy the duration of diabetes was longer (t=3.54; P=0.001), creatinine clearance was lower (t=2.42, P=0.018), triglycerides and fibrinogen levels were higher (t=2.00, P=0.049; t=2.07, P=0.042, respectively). No statistically significant difference was observed between Lp (a) levels, and rates or means of other variables (P > 0.05) (Table 4).

Twenty-two percent of the patients had neuropathy. The duration of diabetes was longer (t=3.01, *P*=0.004), triglyceride levels were higher (t=2.86, *P*=0.005), HDL-cholesterol levels were lower (t=2.41, *P*=0.019), and the ratio of Total cholesterol/HDL cholesterol were higher in patients with neuropathy (t=2.08, *P*=0.19). There was no statistically significant difference between Lp (a) levels, and rates or means of the other variables (*P*>0.05) (Table 5).

DISCUSSION

Diabetes mellitus increases cardiovascular morbidity and mortality by causing premature vascular disease[3, 6]. This increase could not have been explained with the classical risk factors like arterial hypertension, dyslipidemia, obesity and smoking[6]. These findings directed researches to perform prospective and case-control studies to detect new risk factors like Lp (a) in order to prevent diabetes-related microvascular complications.

In this study, we investigated gender dependency of Lp (a) levels by dividing subjects into male and female. Statistical analysis of data showed no statistically significant difference in Lp (a) levels between genders. Previous studies[7-10] on gender dependency of Lp (a), also did not report any gender effect on Lp (a) levels.

Table 3: Statistical evaluation of the data of patients with and without microalbuminuria

Data	Patients with microalbminuria n=26	Patients without microalbuminuria n=45	Statistical Evaluation	P
Men/women ratio	9/17	13/32	X^2=0.253	0.615
Mean age (years)	59.92 ± 8.63	54.82 ± 9.08	t=-2.302	0.023*
Duration of diabetes (months)	7.25 ± 2.62	5.93 ± 2.91	t=-1.901	0.061
BMI (kg/m^2)	29.61 ± 3.39	29.16 ± 3.86	t=-0.492	0.624
HbA1c (%)	8.66 ± 2.03	7.85 ± 2.05	t=-1.602	0.114
Creatinine clearance (mL/min)	108.28 ± 36.70	115.18 ± 33.31	t=0.811	0.420
Total cholesterol (mg/dL)	206.34± 50.05	213.11 ± 45.94	t=-0.578	0.565
Triglyceride (mg/dL)	185.53 ± 133.61	153.86 ± 77.28	t=-1.268	0.209
HDL-cholesterol (mg/dL)	44.07± 14.06	44.86 ± 10.53	t=0.269	0.789
LDL-cholesterol (mg/dL)	121.57 ± 29.78	137.44 ± 40.64	t=1.737	0.087
Total Chol/HDL.cholesterol	4.92 ± 1.67	4.90 ± 1.21	t=-0.066	0.948
BUN (mg/dL)	16.69 ± 4.74	16.11 ± 5.07	t=0.476	0.636
Creatinine (mg/dL)	0.77 ± 0.18	0.75 ± 0.15	t=0.554	0.581
Uric acid (mg/dL)	5.33 ± 1.52	4.63 ± 1.30	t=-2.063	0.043*
Lp (a) (mg/dL)	39.30 ± 35.47	37.80 ± 29.21	t=0.193	0.848
Smoking ratio	12 (% 46.2)	12 (% 26.7)	X^2=2.797	0.094

* P < 0.05 significant, ** P < 0.01 highly significant.

Table 4: Statistical evaluation of the data according to the retinopathy

Data	Patients with microalbminuria n=19	Patients without microalbuminuria n=52	Statistical Evaluation	P
Men/women ratio	5/14	17/35	X^2=0.265	0.607
Mean age (years)	56.47 ± 10.20	56.76 ± 8.91	t=0.119	0.906
Duration of diabetes (months)	8.26 ± 2.32	5.74 ± 2.75	t=-3.548	0.001**
BMI (kg/m2)	29.15 ± 3.74	29.39 ± 3.69	t=0.238	0.813
HbA1c (%)	8.84 ± 1.77	7.89 ± 2.12	t=-1.728	0.088
Creatinine clearance (ml/min)	96.75 ± 30.90	118.47 ± 34.16	t=2.429	0.018*
Total cholesterol (mg/dL)	219.42 ± 49.26	207.42 ± 46.56	t=-0.947	0.347
Triglyceride (mg/dL)	204.68 ± 46.32	151.13±76.74	t=-2.004	0.049*
HDL-cholesterol (mg/dL)	44.78 ± 11.02	44.50 ± 12.24	t=-0.090	0.928
LDL-cholesterol (mg/dL)	133.57 ± 38.49	130.92 ± 37.63	t=-0.262	0.794
Total Chol/HDL.cholesterol	5.09 ± 1.53	4.84 ± 1.34	t=-0.676	0.501
BUN (mg/dL)	17.36 ± 6.69	15.94 ± 4.12	t=-1.080	0.284
Creatinine (mg/dL)	0.74 ± 0.15	0.76 ± 0.17	t=-0.320	0.750
Uric acid (mg/dL)	5.31 ± 1.56	4.73 ±1.34	t=-1.517	0.134
Lp (a) (mg/dL)	28.64 ± 20.28	41.89 ±4.07	t=1.591	0.116
Smoking ratio	5 (26.3%)	19 (36.5%)	X2=0.650	0.420

P < 0.05 significant, ** P < 0.01 highly significant

Table 5: Statistical evaluation of the data according to the presence and absence of neuropathy

Data	Patients with microalbminuria n=43	Patients without microalbuminuria n=28	Statistical Evaluation	P
Men/women ratio	16/27	6/22	?2=1.975	0.160
Mean age (years)	56.46 ± 8.87	57.03 ± 9.82	t=0.254	0.800
Duration of diabetes (months)	7.19 ± 2.56	5.21 ± 2.92	t=-3.012	0.004**
BMI (kg/m2)	29.25 ± 4.03	29.44 ± 3.12	t=0.212	0.833
HbA1c (%)	8.40 ± 2.09	7.76 ± 2.01	t=-1.270	0.208
Creatinine clearance (mL/min)	112.51 ± 36.00	112.88 ± 32.69	t=0.044	0.965
Total cholesterol (mg/dL)	208.23 ± 47.49	214.32 ± 47.49	t=0.528	0.599
Triglyceride (mg/dL)	192.09 ± 115.04	124.57 ± 58.51	t=-2.868	0.005**
HDL-cholesterol (mg/dL)	41.93 ± 11.33	48.64 ± 11.66	t=2.411	0.019*
LDL-cholesterol (mg/dL)	127.97 ± 39.81	137.25 ± 33.86	t=1.016	0.313
Total Chol/HDL cholesterol	5.18 ± 1.43	4.49 ± 1.23	t=-2.081	0.041*
BUN (mg/dL)	16.20 ± 4.51	16.50 ± 5.60	t=0.241	0.810
Creatinine (mg/dL)	0.76 ± 0.17	0.75 ± 0.15	t=-0.210	0.834
Uric acid (mg/dL)	4.84±1.39	4.95±1.47	t=0.312	0.756
Lp (a) (mg/dL)	35.24 ± 28.19	43.12 ± 35.80	t=1.033	0.305
Smoking ratio	17 (39.5%)	7 (25.0%)	?2=1.601	0.206

* P < 0.05 significant ** P < 0.01 highly significant

Considering the complications developed during the course of diabetes, relationship between these complications and Lp (a) levels have been investigated in many studies. In-vitro studies showed that Lp (a) plays a direct role in atherogenesis via cholesterol uptake, or an indirect role via inhibiting fibrinolysis[4].

Guilausseau *et al.,* and colleagues investigated the association between nephropathy and Lp (a). This study indicated that patients having chronic renal failure with or without diabetes (type 1 and type 2 diabetes mellitus) have high Lp (a) levels [11]. In another study which is done by Kopelrud *et. al*, is reported that among patients with type 1 *Diabetes mellitus*, Lp (a) levels were two folds higher in patients with microalbuminuria as well, compared to those without microalbuminuria[12]. However, some conflicting results have also been reported by other studies. Ritter et al suggested that there were no relationship between nephropathy and Lp (a) levels[9]. We also did not find any statistically significant relationship between Lp (a) levels and microalbuminuria (Table 3).

Fifty-six percent of the patients had hypertension in this study. There was no statistically significant difference in Lp (a) levels between patients with and without hypertension. Our results are similar to the results of Heller et al and Nakata et al, who reported no significant association between Lp (a) levels and arterial blood pressure[7,10].

There are several studies in the literature investigating relationship between serum Lp (a) levels and retinopathy. Although significant relationships between serum Lp (a) levels and retinopathy were reported in some of these studies, the rest did not demonstrate such a relationship. Ritter et al reported that serum Lp (a) levels were significantly higher in patients with type 2 diabetes mellitus with comorbid retinopathy, compared to those without retinopathy[9]. In this present study, we did not find any significant relationship between serum Lp (a) levels and retinopathy (Table 4). However, there was a statistically significant relationship between the duration of diabetes and retinopathy. As a result of logistic regression analysis, which was performed to assess the significance level of the parameters effecting

development of retinopathy, duration of diabetes was found to have the greatest effect on retinopathy.

Glycemic control in diabetic patients is followed up by HgbA1c and fructosamine. HgbA1c represents glycemic control for the last 3 months and fructosamine represents for the last 3 weeks. The relationship between Lp (a) and HgbA1c in patients with diabetes was investigated in several studies. Nakata et al and O'Brien et al did not report any association between levels of Lp (a) and HgbA1c in type 2 diabetics[10, 13]. Heller *et al,* Guillausseau *et al,* and Ritter *et al.,* also reported no significant relationship between HgbA1c and Lp (a) levels in patients with both type 1 and type 2 diabetes mellitus[7,9,10]. In accordance with the previous reports, we did not detect any significant correlation between serum levels of HgbA1c and Lp (a) in patients with type 2 diabetes.

Relationship between diabetes and nervous system is known for many years. In literature, its suggested that peripheral neuropathy could develop as a result of diabetes mellitus[14,15]. Diabetic neuropathy was classified as: sensory or sensory motor polyneuropathy, autonomic neuropathy, motor neuropathy of symmetrical proximal lower extremity, cranial neuropathy, body radiculopathy or mononeuropathy, extremity plexus or mononeuropathy, multiple mononeuropathies, ischemic nerve injury, asymmetric neuropathy, and mixed forms with distal symmetric polyneuropathy. Neuropathy was confirmed in 22 % of the study patients. Although statistical analyses revealed no significant correlation between Lp (a) and diabetic neuropathy, in patients with neuropathy the duration of diabetes was longer, triglyceride levels were higher and HDL cholesterol levels were lower (Table 6, Fig. 6). It is demonstrated that diabetes mellitus is a syndrome initially characterized by a loss of glucose homeostasis. In literature the levels of glucose have been increased significantly for 1, 3 and 6 h, while levels of cholesterol decreased significantly in different time periods in vivo in 1, 3 and 6 h (in all time periods)[16].

In conclusion, when we compared diabetic patients with and without chronic microvascular complications, there was no statistically significant difference between Lp (a) levels.

Results of logistic regression analysis, which was performed to assess the significance level of the parameters effecting development of neuropathy indicated that triglycerides had the greatest effect on neuropathy by increasing neuropathy risk 1.19 times.

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