Original Article

Lipoprotein(a) in South Indian Type 2 Diabetic Subjects in Relation to Diabetic Vascular Complications

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Abstract

- **Objectives : Lipoprotein(a) [LP(a)] has been reported to be an independent risk factor for coronary artery disease (CAD). However, its relationship with other vascular complications is not clear. The aim of the study was to determine the relation of lipoprotein(a) with micro- and macrovascular complications seen in type 2 diabetic patients.**
- **Methods : We studied 725 type 2 diabetic patients with and without diabetic complications at the MV Diabetes Specialities Centre, Chennai. The mean age of the study group was 54 ± 10 years and 70% were males. Diabetic complications viz retinopathy, proteinuria, peripheral vascular disease and coronary artery disease were diagnosed using standardized definitions. Lipoprotein(a) levels were measured using enzyme linked immunosorbant assay (ELISA). Since the frequency distribution of Lp(a) was skewed Lp(a) values were log transformed and geometric mean was used for statistical analysis.**
- **Results : The mean Lp(a) level of patients with any vascular complication was significantly higher compared to the subjects without any complications. Multiple logistic regression analysis revealed that lipoprotein(a) had as independent association with CAD (Odds Ratio - 1.16, p=0.04) and proteinuria (Odds Ratio - 1.69, p < 0.001). The association of Lp(a) with retinopathy and PVD turned out to be non-significant when CAD and proteinuria was introduced as cofactors in the regression model.**
- **Conclusion : Lp(a) concentrations are found to be higher in those with CAD and proteinuria. There appears to be no association between Lp(a) and retinopathy or PVD in South Indian type 2 diabetic patients.**

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Introduction

Lipoprotein(a) [Lp (a)] is a form of low density lipoprotein (LDL) and has an apoprotein (a) [apo(a)] molecule covalently linked to apoprotein (B) [apo B 100]. Lp(a) is genetically determined and has a structural homology similar to plasminogen.[1] Lp(a) binds to fibrin and to membrane proteins of endothelial cells and monocytes and thereby inhibits plasmin generation.[1] This could lead to atherogenesis and thrombogenesis.[1] Serum Lp(a) is an independent risk factor for coronary artery disease (CAD).[1]

The risk for coronary artery disease is higher among diabetic subjects as compared to non-diabetic subjects.[2] This increased risk for CAD among diabetic subjects is only partially explained by increased conventional risk factors.[2] According to the recent WHO statistics, India tops the world with the largest number of diabetic subjects in any given country and this trend is expected to increase.[3] In an earlier study, we reported that Lp(a) is an independent risk factor for CAD among South Indian type 2 diabetic patients.[4] The association of Lp(a) with other diabetes related complications is unclear. The aim of the present study was to determine the association of Lp(a) with diabetes related vascular complications in South Indian type 2 diabetic subjects.

Research design and methods

Seven hundred and twenty five consecutive type 2 diabetic patients attending the MV Diabetes Specialities Centre at Chennai during the period June '97 to June '98, were recruited for the study. Diagnosis of type 2 diabetes was based on WHO study group criteria,[5] (i.e. fasting plasma glucose ≥ 140 mg/dl or 2 hr post-glucose levels ≥ 200 mg/dl). Clinical examination included recording of height and weight with calculation of body mass index (BMI). A fasting blood sample was taken and serum separated and stored at -20°C until the assays were performed.

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Total serum cholesterol, serum triglyceride and HDL cholesterol (after precipitation of LDL and chylomicrons with phosphotungstic acid and magnesium chloride) were assayed with a commercial kit (Boehringer Mannheim, Germany) using Hitachi - 912 Autoanalyser (Hitachi, Germany). LDL cholesterol was calculated according to the Friedewald equation.[6] Urinary protein was measured on a spot urine by sulfosalicylic acid technique.[7] Lp(a) was estimated by enzyme immunoassay (Macra Lp(a), Strategic Diagnostic, Newark, NJ). Briefly, microtiter plates precoated with monoclonal anti-Lp(a) antibodies were incubated with appropriately diluted serum samples. Bound Lp(a) was measured by incubation with a polyclonal anti-Lp(a) antibody coupled to horseradish peroxidase followed by the substrate hydrogen peroxide. A calibration curve was developed using standards (0-80 mg/dl) provided with the kit. Intra- and interassay coefficients of variation for Lp(a) were 6.6 and 9.3% respectively.

Definitions

Proteinuria

Patients were considered to have proteinuria, if the 24 hour urine protein excretion was ≥ 500 mg on more than two occasions without any evidence of urinary tract infection.

Retinopathy

The ocular fundi were examined by a retinal specialist both by direct and indirect ophthalmoscopy, after mydriasis. Retinopathy when present was classified as Non-Proliferative Diabetic Retinopathy (NPDR) and Proliferative Diabetic Retinopathy (PDR). NPDR was diagnosed when there was evidence of microneurysms, dot haemorrhages, exudates or cotton wool spots in the absence of any new vessels or advanced diabetic eye disease. PDR was diagnosed when any new vessels were present or if there was evidence of fibrous retinitis proliferans, vitreous haemorrhage, retinal detachment or other features of advanced diabetic eye disease. NPDR and PDR were taken together as retinopathy for this study.

Coronary artery disease (CAD) was considered to be present when either myocardial ischaemia for infarction was present.

Myocardial ischaemia was diagnosed if there was a history of exertional chest pain (angina) with unequivocal T wave changes in the electrocardiogram (ECG), but no evidence of infarction.

Myocardial infarction was diagnosed if there was a classical history of chest pain documented by hospital records along with ST or Q wave changes on ECG suggestive of recent or past myocardial infarction.

Peripheral vascular disease (PVD) was diagnosed using Doppler recording of pressure tracings using a KODY Vaslab machine (Kody Labs, Madras). An ankle-brachial pressure index of < 0.8 was considered as evidence of PVD.

Statistical Analysis

Lp(a) levels were transformed into natural logarithms and values were expressed as geometric mean (SD). Student's T-test was used to compare means of continuous variables in two groups. Logistic regression analysis was carried out to determine the association of Lp(a) with diabetes-related complications. CAD, PVD, proteinuria or retinopathy were used as dependant variables. As these diabetes-related complications are interlinked and a strong association of Lp (a) with CAD and proteinuria are well established, different models were constructed to determine the association of Lp(a) with these complications by adjusting for other complications one by one. All analyses were performed with the SPSS statistical software package (version 4.0.1, SPSS, Chicago); p values < 0.05 were considered significant.

Results

Clinical features of the study group are presented in Table 1. Seventy percent of the study group were males. The prevalence of CAD among the study population was 37.8%, PVD 3.5%, proteinuria 15.3% and retinopathy 34.3%.

Table 1 : Clinical features of the study group (n=725)

Variables	
Age (yrs)	54 ± 10
Male	70%
BMI (kg/m^2)	24.3 ± 4.1
Systolic blood pressure (mm Hg)	138 ± 17
Diastolic blood pressure (mm Hg)	84 ± 8
Fasting plasma glucose (mmol/l)	8.4 ± 2.8
HbA_{1c} (%)	9.0 ± 2.1
Serum cholesterol (mmol/l)	5.1 ± 1.2
Serum triglycerides (mmol/l)	2.1 ± 0.1
Lipoprotein (a) (mg/dl)	15.5 (3.2)
CAD n(%)	274 (37.8%)
Proteinuria n(%)	111 (15.3%)
PVD n(%)	25 (3.5%)
Retinopathy n(%)	249 (34.3%)

Lp (a) values are expressed as geometric mean (SD)

Table 2 presents the mean Lp(a) levels in subjects with and without vascular complications. The mean Lp(a) levels of patients with any vascular complications was significantly higher as compared to the subjects without any complications. Subjects with more than two complications (n=181) had significantly higher Lp(a) values [geometric mean (SD) - 21.5 (3.1) mg/dl] compared to subjects with any one complication (n=437, 17.8 (3.1) mg/dl), p = 0.053). This was inturn higher compared to no complication group (n=279, 12.7 (3.2) mg/dl), p < 0.001.

Table 3 presents the results of multiple logistic regression analysis. In model 1, the association of Lp(a) with diabetes related complications such as CAD, PVD, prote-

Table 2 : Mean Lp(a) levels in type 2 diabetic patients with and without diabetic complications

Complications	Present	Absent	p value
Coronary artery disease [mg/dl]	17.8 (3.0) (n=274)	14.2 (3.2) (n=451)	0.009
Peripheral vascular disease [mg/dl]	26.0 (2.9) (n=25)	15.2 (3.2) (n=700)	0.021
Retinopathy [mg/dl]	18.2 (3.2) (n=249)	14.3 (3.1) (n=476)	0.007
Proteinuria [mg/dl]	26.8 (2.7) (n=111)	14.0 (3.2) (n=614)	< 0.0001

Lp(a) values are expressed as geometric mean (SD)

inuria or retinopathy was determined. All the complications had a strong association with Lp(a). In the next model, presence of other diabetic complications were introduced as independent variables one after another. The association of Lp(a) with PVD and retinopathy turned out to be non-significant when nephropathy was introduced as a covariate into the model. Lp(a) had a strong association with CAD ($p = 0.04$) and proteinuria ($p < 0.01$) even after the introduction of all the other variables into the model. Proteinuria had a strong association with retinopathy ($p < 0.001$). Retinopathy ($p = 0.004$) and PVD ($p = 0.006$) had a strong association with CAD.

Discussion

Elevated Lp(a) levels are considered to be an independent risk factor for CAD.[4,8] Though diabetic subjects have been reported to have higher risk of developing CAD,[2] the relationship of Lp(a) with diabetes per se is still not clear [8,9]

Levitsky *et al*[10] reported an increased Lp(a) concentration in patients with type 1 diabetes mellitus and Bruckert *et al* reported that Lp(a) concentrations are related to the degree of glycemic control.[11] On the other hand, studies have also reported no association between Lp(a) concentrations and the degree of glycemic control.[12]

Similarly, contradictory results have been reported with regard to type 2 diabetes and Lp(a) levels, with some reporting increased Lp(a) levels, while some others show no significant difference in Lp(a) levels in diabetic individuals as compared to healthy normals. Certain studies even report that Lp(a) levels are lower in diabetic patients without complications as compared to normal control subjects.[8] Thus the relationship of Lp(a) with diabetes is far from clear and a proper association is yet to be determined.

With regard to diabetic complications, the situation is even less clear as the available reports are highly contradictory and this topic has been reviewed recently.[8] We therefore performed this study, in order to elucidate the relationship of Lp(a) with diabetic complications in south Indian diabetic subjects and to our knowledge this is the first report from our country on this topic and indeed one of the first comprehensive reports on Lp(a) with respect to all diabetic complications.

High prevalence of CAD despite low rates of conventional risk factors has been reported frequently in Asian Indians.[2] Recent reports suggest that Lp(a) is a strong independent risk factor for CAD.[4,8] Furthermore comparative studies have revealed that Indians have very high levels of Lp(a) as compared to other ethnic populations.[13] The present study corroborates earlier findings that Lp(a) is strongly associated with CAD in Indians.

PVD is another macrovascular complication reported to be very common among diabetic subjects. However, atherosclerosis of the lower extremities in diabetic patients is rare in South Indian type 2 diabetic patients.[14] The reason for the low prevalence of PVD and high prevalence of CAD remains a paradox. Cheng *et al*[15] reported a strong association between Lp(a) and PVD in diabetic as well as non-diabetic subjects. In the present study, a higher level of Lp(a) was noted in patients with PVD. However, this association turned out to be non-significant with the inclusion of proteinuria and other diabetic complications into the logistic regression model. This is in agreement with the findings of O'Neal *et al*,[16] that there was no difference in the apoprotein(a) concentration in diabetic patients with and without PVD.

Lp(a) being an antifibrinolytic agent could lead to occlusion of blood vessels. However, not many studies have explored the relationship of Lp(a) with microvascular disease. In the present study, we also looked at the association of Lp(a) with microvascular complications namely retinopathy and nephropathy. Mean Lp(a) levels of patients in the retinopathy group was higher than those in the non-retinopathy group. However with inclusion of proteinuria as an independent variable into the logistic regression model, the association of Lp(a) with retinopathy disappeared. Thus, the increase in Lp(a) in the retinopathy group could be explained by the strong association of Lp(a) with proteinuria[17] which in turn is known to have a strong link with diabetic retinopathy.[18] Earlier studies on Japanese diabetic patients revealed a positive correlation of serum Lp(a) levels with retinopathy.[19] On the con-

Table 3 : Multiple logistic regression analysis

	Model 1		Model 2		Model 3		Model 4	
	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
Dependent variable : CAD								
Independent variables:								
Lp(a)	1.19 (1.04-1.36)	0.009	1.19 (1.04-1.37)	0.011	1.18 (1.03-1.4)	0.016	1.16 (0.006-1.33)	0.04
Proteinuria	—		0.98 (0.64-1.5)	0.93	0.83 (0.53-1.3)	0.41	0.82 (0.52-1.3)	0.37
Retinopathy	—		—		1.6 (1.2-2.3)	0.004	1.6 (1.16-2.26)	0.004
PVD	—		—		—		3.3 (1.41-7.9)	0.006
Dependent variable : PVD								
Independent variables:								
Lp(a)	1.61 (1.07-2.4)	0.021	1.5 (0.99-2.3)	0.054	1.48 (0.98-2.23)	0.063	1.42 (0.9-2.2)	0.10
Proteinuria	—		2.13 (0.87-5.2)	0.098	1.8 (0.71-4.6)	0.22	1.87 (0.73-4.8)	0.19
Retinopathy	—		—		1.65 (0.71-3.8)	0.25	1.48 (0.64-3.5)	0.36
CAD	—		—		—		3.3 (1.41-7.9)	0.006
Dependent variable: Proteinuria								
Independent variables:								
Lp(a)	1.8 (1.4-2.2)	< 0.0001	1.68 (1.36-2.08)	< 0.0001	1.69 (1.36-2.1)	< 0.0001	1.69 (1.36-2.1)	< 0.0001
Retinopathy	—		4.1 (2.6-6.3)	< 0.0001	4.13 (2.67-6.39)	< 0.0001	4.03 (2.6-6.2)	< 0.0001
CAD	—		—		0.85 (0.54-1.32)	0.47	0.84 (0.53-1.3)	0.43
PVD	—		—		—		1.9 (0.76-4.96)	0.17
Dependent variable: Retinopathy								
Independent variables:								
Lp(a)	1.21 (1.06-1.4)	0.0064	1.11 (0.96-1.23)	0.17	1.08 (0.94-1.25)	0.28	1.08 (0.94-1.25)	0.27
Proteinuria	—		4.1 (2.6-6.3)	< 0.0001	4.18 (2.7-6.47)	< 0.0001	4.07 (2.63-6.3)	< 0.0001
CAD	—		—		1.63 (1.17-2.25)	0.0036	1.64 (1.16-2.26)	0.004
PVD	--		—		—		1.44 (0.61-3.4)	0.40

Continuous variable : natural logarithm of Lp(a), Discrete variables : Proteinuria, Retinopathy, CAD and PVD.
Presence of complication = 1, absence of complication = 0.

trary, the WESDR study[20] has reported absence of an association of Lp(a) with diabetic retinopathy.

The Lp(a) levels were high among patients with proteinuria as compared to subjects without proteinuria in this study. The statistical significance of this association was retained even after the inclusion of all the other diabetic complications into the logistic regression model. These results are in agreement with Kronenberg *et al.*[17] The strong association of Lp(a) with overt proteinuria could probably be due to the decreased excretion of Lp(a) in patients with renal failure.

In conclusion, in this clinic based study of South Indian type 2 diabetic patients, Lp(a) concentrations are

found to be higher in those with coronary artery disease and nephropathy. There appears to be no association with retinopathy or PVD. Prospective studies are needed to elucidate the exact role of Lp(a) in diabetic vascular complications.

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