Lipoprotein (a) and vascular disease in diabetic patients

R. W. James¹, M. Boemi², C. Sirolla³, L. Amadio³, P. Fumelli², D. Pometta¹

¹ Division of Diabetology, University Hospital, Geneva, Switzerland

² Division of Diabetology, INCRCA, Ancona, Italy

³ Department of Demographic and Statistical Studies, INRCA, Ancona, Italy

Summary In order to assess the potential role of lipoprotein (a) as a risk factor for cardiovascular disease in diabetes mellitus, plasma concentrations were measured in a large group ($n = 500$) of non-insulin-dependent (NIDDM, $n = 355$) and insulin-dependent (IDDM, $n = 145$) patients. Concentrations of lipoprotein (a) were compared in diabetic patients with ($n = 153$) or without (347) documented vascular disease (ischaemic heart disease, peripheral vascular disease or macroangiopathy). They were significantly higher ($p < 0.05$) in patients with ischaemic heart disease (mean [interquartile range] 15.5 (5.0–38.0) vs 9.0 (4.5–26.0) mg/dl) or macroangiopathy (13.0 (5.0–

38.0) vs 9.0 (4.0–25.0) mg/dl) compared to patients without manifestations of vascular disease. In addition, stepwise logistic regression analysis identified lipoprotein (a) levels ≥ 30 mg/dl as being independently associated with the presence of cardiovascular disease. Lipoprotein (a) was an independent risk factor for ischaemic heart disease and macroangiopathy in this group of IDDM and NIDDM patients. [Diabetologia (1995) 38: 711–714]

Key words Lipoprotein (a), vascular disease, risk factor, lipoproteins, apolipoproteins.

Lipoprotein (a) (Lp(a)) is a form of low density lipoprotein (LDL) modified by the covalent binding of a large glycoprotein, apolipoprotein (a), to the structural apolipoprotein (apo) of LDL, apo B. [1]. It has aroused clinical interest in the non-diabetic population due to suggestions that it is a cardiovascular disease (CVD) risk factor independent of conventional lipid/lipoprotein parameters [2, 3]. This contention is supported by the majority of available studies [3–7] with some dissenting opinion [8].

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Corresponding author: Dr. R. W. James, Division of Diabetology, Department of Medicine, University Hospital, 24 Rue Micheli-du-Crest, CH-1211 Geneva 14, Switzerland

Abbreviations: Lp(a), lipoprotein (a); LDL, low density lipoproteins; CVD, cardiovascular disease; IHD, ischaemic heart disease; PVD, peripheral vascular disease; HDL, high density lipoproteins.

The significance of Lp(a) in diabetes is unclear essentially due to a paucity of relevant studies. A recent, critical analysis by Haffner [9] concluded that available data did not indicate an increase in plasma levels of Lp(a) in non-insulin-dependent (NIDDM) patients, compared to control subjects, but that concentrations may be higher in insulin-dependent (IDDM) patients. Studies of the relationship of Lp(a) to CVD have yielded conflicting conclusions; three reports focusing on NIDDM patients found Lp(a) to be an independent risk factor [10–12] whilst other reports [13–16], including IDDM patients, were unable to show a significant relationship between Lp(a) and vascular disease. Further studies are thus necessary before firm recommendations can be made concerning the clinical relevance of Lp(a) in diabetic subjects. In this context, the present study examined the relationship between plasma Lp(a) concentrations and the prevalence of cardiovascular disease in a large group of IDDM and NIDDM patients.

Patients and methods

Subjects were recruited from those consecutively attending the Diabetes Centre in Ancona Hospital for their routine control visits during 1992–1993. Free medical treatment is available to diabetic subjects under the Italian health system, so that patients attending the centre are representative of the diabetic population in the Ancona region. A total of 500 subjects (258 women, 242 men) were recruited, of whom 145 (75 men, 70 women) were IDDM patients and 355 (183 men, 172 women) were NIDDM patients (83 treated with insulin). Diabetes was diagnosed on the basis of World Health Organization (WHO) criteria.

Vascular disease was determined in the following way. Ischaemic heart disease (IHD) was defined as definite myocardial infarction (hospital-verified), ischaemic electrocardiographic alterations (Q/QS and ST/T changes, bundle branch changes) angina pectoris according to clinical history and conventional resting electrocardiograms (evaluated according to the Minnesota code [17]). Peripheral vascular disease (PVD) included arteriosclerosis obliterans (clinical history of lower limb intermittent claudication, resting ankle:arm blood pressure ratio less than 0.9 or Doppler velocimetry (femoral, popliteal, posterior tibial and dorsalis pedis arteries with a monophasic velocity signal above the zero flow base line considered pathological at any level)) and cerebrovascular disease (documented history of transient ischaemic attack, reversible ischaemic neurological deficit or stroke due to cerebral infarction). Macroangiopathy was defined as IHD and/or PVD. No exercise tests were applied. Hypertension was defined according to WHO guidelines. Subjects were divided into three groups according to their body mass index (BMI, weight (kg)/height (m)²): normal weight less than 25, overweight greater than 25 and less than 29, obese greater than 29.

A fasting blood sample was taken and centrifuged to obtain plasma. Cholesterol, triglycerides and high density lipoprotein (HDL) cholesterol (after precipitation of lower density lipoproteins with heparin-manganese (Wako, Neuss, Germany) were assayed with commercial kits (Beckmann, Milan, Italy). LDL-cholesterol was calculated according to the Friedewald equation [18]. Lp(a) was assayed with enzyme immunoassay (Macra Lp(a), Terumo, Elkton, Md., USA). Briefly, micro-titre plates pre-coated with monoclonal anti Lp(a) antibodies were incubated with appropriately diluted (201:1) plasma samples. Bound Lp(a) was revealed by incubation with a polyclonal antibody against the apo(a) component of Lp(a) 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 inter-coefficients of variation were 3.7–5.8 and 7.4–10.4, respectively.

Statistical analysis

The Student's *t*-test was used to compare means of continuous variables, and the chi-square test for comparison of categories (triglycerides were converted to log values prior to analysis). For Lp(a), the Kolmogorov-Smirnov two-sample test was performed to compare differences between the two groups. Analysis of variance (ANOVA) was applied to evaluate the lipid profile according to the presence or absence of CVD and as a function of Lp(a), either less than 29.9 or more than 30 mg/dl [19], adjusting for age, sex, duration and type of diabetes. Logistic regression analysis was used to identify associations between CVD and the independent parameters. Model building was performed by forward, stepwise selection using the likeli-

Table 1. Demographic characteristics of IDDM and NIDDM patients

	IDDM	NIDDM
<i>n</i>	145	355
Age (years)	36.5 ± 14.4	63.2 ± 10.3 ^a
Duration (years)	14.9 ± 10.6	13.5 ± 9.2
Triglycerides (mmol/l)	1.28 ± 0.86	1.77 ± 1.06 ^a
Cholesterol (mmol/l)	5.00 ± 1.09	5.73 ± 1.30 ^a
LDL-cholesterol (mmol/l)	3.47 ± 0.93	4.00 ± 1.15 ^a
HDL-cholesterol (mmol/l)	0.94 ± 0.39	0.94 ± 0.43
Lp(a) (mg/dl)	10.0 (4.5–22.7)	10.0 (4.5–33.0)
HbA _{1c} (%)	8.7 ± 1.7	8.2 ± 1.2 ^b
BMI	23.9 ± 3.7	27.1 ± 4.2 ^a

Mean values (± SD) are given except for Lp(a) (median (interquartile range)). Triglycerides were converted to log values prior to analysis. ^a *p* < 0.001, ^b *p* < 0.05 vs IDDM patients

Table 2. Plasma lipid, apolipoprotein and HbA_{1c} levels in diabetic patients with and without macroangiopathy

	Macroangiopathy	
	Absent	Present
<i>n</i>	347	153
Triglycerides (mmol/l)	1.52 ± 0.92	1.89 ± 1.21
Cholesterol (mmol/l)	5.37 ± 1.21	5.84 ± 1.39
LDL-cholesterol (mmol/l)	3.74 ± 1.06	4.08 ± 1.20
HDL-cholesterol (mmol/l)	0.95 ± 0.40	0.93 ± 0.45
Lp(a) (mg/dl)	9.0 (4.0–25.0)	13.0 (5.0–38.0) ^a
HbA _{1c} (%)	8.3 ± 2.0	8.4 ± 1.9

Mean values (± SD) except for Lp(a) (median (interquartile range)) in diabetic patients with (present) and without (absent) macroangiopathy. Triglycerides were converted to log values prior to analysis. ^a *p* < 0.05 vs patients without macroangiopathy. All other comparisons were not significant

hood ratio to remove variables. All analyses were performed with the SPSS statistical software package (version 5.0, SPSS Inc., Chicago, Ill., USA); probability values less than 0.05 were accepted as significant.

Results

Demographic characteristics of the IDDM and NIDDM patients are given in Table 1. In the combined population, 103 patients (20.6%) had IHD, 93 patients (18.5%) presented with PVD whilst macroangiopathy was diagnosed in 153 patients (30.6%). Hypertension was present in 189 patients (37.8%). With respect to Lp(a), plasma concentrations were less than 30 mg/dl in 127 patients (25.4%) of whom 24 (16.6%) were IDDM and 103 (29.9%) were NIDDM (*p* < 0.01 comparing IDDM with NIDDM patients with respect to elevated Lp(a) levels). Patients with vascular disease were older and had diabetes of longer duration (*p* < 0.01). Patients with macroangiopathy also had a greater BMI (*p* < 0.05). Table 2 gives the comparisons of lipid levels between patients with and without vascular disease, using macroangiopathy as an example. After

Table 3. Logistic regression analysis of determinants of vascular disease: final models

	B	Odds ratio	p value
<i>Ischaemic heart disease</i>			
Constant	-6.0486		
Age	0.0579	1.06	< 0.001
Insulin treatment	0.6755	1.97	0.007
Hypertension	1.3426	3.83	< 0.001
Lp(a) $\geq$ 30 mg/dl	0.6512	1.92	0.016
<i>Peripheral vascular disease</i>			
Constant	-4.8218		
Age	0.0436	1.04	< 0.001
Duration	0.0301	1.03	0.015
Hypertension	0.5966	1.81	0.017
<i>Macroangiopathy</i>			
Constant	-5.1928		
Age	0.0516	1.05	< 0.001
Duration	0.0354	1.04	0.002
Hypertension	1.2887	3.63	< 0.001
Lp(a) $\geq$ 30 mg/dl	0.7459	2.11	0.003

For logistic regression analysis, IHD, PVD and macroangiopathy were dependent variables; independent variables were age, sex, triglycerides, cholesterol, LDL-cholesterol, HDL-cholesterol, Lp(a) (≤ 29.9 or ≥ 30.0 mg/dl), BMI (coded as normal, overweight or obese), hypertension, insulin treatment, type and duration of diabetes

adjusting for age, sex, BMI, duration and type of diabetes, no significant differences in cholesterol, LDL-cholesterol, HDL cholesterol or triglyceride levels were observed, although cholesterol and triglyceride levels tended to be higher in affected patients. Neither did HbA_{1c} values differ significantly between the two groups (Table 2) or between patients with and without cardiovascular disease when considered according to the type of diabetes (results not shown). Lp(a) concentrations were, however, significantly higher in patients with macroangiopathy. Similar results (not shown) were obtained when patients with IHD or PVD were considered separately. That is, no significant differences in concentrations of conventional lipid risk factors: Lp(a) levels were higher in patients with IHD (median (interquartile range) mg/dl; 15.5 (5.0–38.0) vs 9.0 (4.5–26.0)) ($p < 0.05$) and PVD (13.0 (5.0–41.0) vs 10.0 (4.0–28.0)), not significant). There were no significant differences (chi-square test) in the use of lipid-lowering drugs between patients without or with macroangiopathy (7.8 vs 10.5 % of subjects), IHD (7.8 vs 11.7 %) and PVD (7.9 vs 11.8 %).

Logistic regression analysis was employed to determine which factors best predicted the occurrence of vascular disease. As shown in Table 3, Lp(a) ≥ 30 mg/dl [19] emerged as a significant predictor of IHD and macroangiopathy, but not of PVD. None of the other conventional lipid risk factors were significant predictors of vascular disease in this group of Italian, diabetic patients. To illustrate the influence of Lp(a) ($<$ or > 30 mg/dl) Figure 1 shows the prob-

Fig. 1. The probability of developing macroangiopathy with age for subjects with hypertension, 10–20 years duration of diabetes and Lp(a) less than 30 mg/dl (—) or greater than 30 mg/dl (-----)

ability of developing macroangiopathy with age for subjects with hypertension and 10–20 years duration of diabetes.

Discussion

The present study indicates that Lp(a) is associated with the presence of vascular disease irrespective of the type of diabetes. When examined as a continuous variable, plasma concentrations were significantly higher in diabetic subjects with IHD and macroangiopathy, whilst Lp(a) was independently associated with IHD and macroangiopathy as a dichotomous variable (Lp(a) < 30 or ≥ 30 mg/dl).

Given the small number of studies available pertaining to the topic, it is presently difficult to offer coherent explanations for the conflicting results concerning the relationship between CVD and Lp(a) in diabetes. Factors such as the definition of CVD and plasma cholesterol concentrations could play a role. It has recently been suggested that Lp(a) may be increased in the presence of raised cholesterol levels [7] but this argument cannot be invoked in the context of studies of diabetic subjects. Cholesterol levels were similar or even lower in studies where Lp(a) emerged as a risk factor compared to studies where no impact on vascular disease was apparent [10–16]. It is probable that the number of patients is an important consideration. In this respect, it should be noted that the present study represents one of the largest, in terms of number of patients, that has examined Lp(a) as a risk factor in diabetes. As underlined by Csaszar et al. [20], the highly skewed distribution of Lp(a) plasma concentrations can give rise to misleading conclusions where small numbers of patients are involved.

A feature of the present study was the particularly strong association between hypertension and cardiovascular disease in this population. In contrast, the conventional lipid and apolipoprotein factors did not

emerge as being independently related to vascular disease. This could not be attributed to the use of lipid-lowering drugs. These results mirror previous studies of Lp(a) in diabetic patients, where lipids/apolipoproteins also failed to emerge as being independently associated with cardiovascular disease [11, 13].

The question of whether diabetes itself increases Lp(a) levels was not addressed in the present study. It should be noted, however, that a significantly greater proportion of NIDDM patients had Lp(a) values above 30 mg/dl. The consensus opinion [9], albeit based on a limited number of studies, is that NIDDM does not increase Lp(a), whilst the question remains open for IDDM. We were unable to examine whether renal dysfunction could influence Lp(a) levels. The impact of nephropathy on Lp(a) in diabetes is, however, far from clear [9]. The majority of studies do not indicate an effect in NIDDM [9, 10, 12, 21, 22], whilst an equal number of reports support [23–25] or dismiss [15, 26, 27] an impact of renal dysfunction on Lp(a) in IDDM. Further studies are evidently necessary.

In conclusion, Lp(a) concentrations greater than 30 mg/dl were independently associated with vascular disease in this group of IDDM and NIDDM patients. These results argue in favour of more aggressive treatment of modifiable risk factors in diabetic patients with high plasma concentrations of Lp(a).

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