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Lipids, lipoprotein (a) and coronary artery disease in patients following cardiac transplantation

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Abstract Cardiac allograft vascular disease (CAVD) is the most important cause of late mortality in cardiac transplant recipients. While the pathogenesis of the disease is believed to be immunological, other factors like hyperlipidaemia may contribute. Total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, apolipoprotein A1 and B and Lp(a) levels were measured in 174 cardiac transplant recipients attending our clinic for routine follow-up. Univariate and multivariate logistic regression analysis was carried out to assess the relationship of the variables studied to the presence of CAVD diagnosed with coronary angiography. CAVD was present in 42 of the 174 patients. The group with CAVD had a higher total cholesterol (6.8 vs 6.3 mmol/l), lower HDL cholesterol (0.8 vs 0.9 mmol/l), higher triglyceride (2.8 vs 2.0 mmol/l) and higher Lp(a) level (317.5 vs 95 mg/l) than the group without CAVD. In multivariate analysis, after adjusting for gender, hypertension, time from transplantation, preoperative diagnosis and lipid-lowering therapy, Lp(a), total cholesterol, HDL cholesterol and triglycerides remained significantly correlated with CAVD. The results indicate a significant association between hyperlipidaemia, Lp(a) levels and allograft vascular disease. Further studies are needed to show whether treatment of hyperlidaemia in this population delays the onset or slows the progression of CAVD.

Key words Heart transplantation, lipids, coronary disease · Lipids, heart transplantation, coronary artery disease · Coronary artery disease, heart transplantation, lipids

Introduction

Cardiac allograft vascular disease (CAVD) has been a recognised complication of heart transplantation in animal experiments [17] and was also described in the early years of human transplantation [4, 29]. While there has been a marked improvement in the survival of these patients with the advent of cyclosporin-based immunosuppression, the incidence of CAVD has not changed significantly [11] and is believed to affect 5 %–10 % of recipients each year [23, 30]. The contribution of immunological factors to the aetiology of CAVD has been emphasised recently [24, 26], but it is likely that other factors also contribute. The effects of age, sex, obesity, hypertension, smoking, diabetes mellitus, pre-transplant diagnosis, donor age and sex have been reported in numerous studies with variable results [5, 10, 27]. Some investigators have found an association with cytomegalovirus infection [14, 20]. Several studies have reported a correlation between hyperlipidaemia and accelerated coronary artery disease [8, 32]. Recently, Barbir et al. reported that a high concentration of serum lipoprotein(a) [Lp(a)] was an independent risk factor for the development of this condition [2].

The aim of this study was to examine the relationship of serum lipids and Lp(a) with the development of coronary artery disease in a group of cardiac transplant recipients.

Patients and methods

During a 6-month period, serum cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), apolipoprotein A1 (apo A1), apolipoprotein B (apo B) and Lipoprotein (a) [Lp(a)] levels were measured in 174 patients who attended our transplant clinic for routine follow-up. Coronary angiography was performed in all patients within 6 months of the measurement of the above variables. Patients were not treated with nitroglycerin or other vasodilators during coronary angiography. Allograft vascular disease was defined as the presence of any degree of stenosis in any part of the coronary tree on coronary angiography.

Standard enzyme methods were used to measure total cholesterol and triglycerides. HDL was measured after separation by tungstophosphoric acid and magnesium chloride precipitation. LDL was calculated by the Friedwald formula. Lp(a) was measured using the Immunozym Lp(a) assay (Immuno, Sevenoaks, Kent, UK). The Immunozym Lp(a) assay is a one-step sandwich ELISA in which Lp(a) particles and free apo(a) are bound to the solid phase during the first incubation step. The concentration of bound Lp(a) and free apo (a) is determined by colour change at 450 nm following washing and a second stage of incubation. Lp(a) is measured in this assay with a precision of 5.3 %, the detection limit being 10 mg/l. Apo A1 and apo B were measured by immunoturbidimetry using commercially available kits (Instrumentation Laboratory, Warrington, Cheshire, UK). In these assays the apolipoprotein reacts with a specific antibody in the presence of PEG buffer, producing a suspension of antigen-antibody complexes with the resultant increase in turbidimetry being measured at 340 nm. The precision of apo A1 and apo B measurements was 2.2 % in each case; the lowest concentration detectable in these assays was 0.45 g/l and 0.32 g/l, respectively.

Results are expressed as mean (SD) or median (range), as appropriate. For continuous variables, the Mann-Whitney U-test was used to determine the statistical significance of the difference between the two groups. As Lp(a) and triglyceride levels were not normally distributed, the Mann-Whitney U-test was used to determine the statistical significance of differences between the two groups. For consistency this test was used for all continuous variables. The chi-square test for contingency tables was used for categorical variables. A P value less than 0.05 was considered statistically significant. Multivariate logistic regression analysis was carried out to assess the association between the variables studied and the presence of allograft vascular disease. Results were adjusted for time since transplantation, gender, hypertension and a pre-operative diagnosis of ischaemic heart disease since these have been associated with CAVD in the past. Log transformations were carried out where necessary and P values are given for the likelihood ratio test and the Wald test.

Results

The 174 patients studied were divided into two groups on the basis of coronary angiography: group 1 (132 patients) had normal angiograms while group 2 (42 patients) had evidence of allograft vascular disease. All but one patient had received cyclosporin-based immunosuppression. Fifty patients were on prednisolone at the time of the analysis, 37 in group 1 and 13 in group 2. The mean dose of prednisolone was 8.9 mg/day in group 1 and 8.8 mg/day in group 2. Fifty-six patients were on lipid-lowering therapy (either a fibrate, simvastatin or pravastatin), 36 in group 1 and 20 in group 2. Tables 1 and 2 show the summaries of the variables studied in the two groups and the results of the Mann-Whitney and chi-square tests.

Table 1 Distribution of variables examined in the two groups

Variable	Group 1 – no CAVD	Group 2 – CAVD	P
Mean time since transplant (SD)	57 (27) months	67 (30) months	0.041
Mean age (SD)	49 (11) years	52 (10) years	0.111
Mean age donor (SD)	27 (10) years	29 (11) years	0.170
Recipient gender (F/M)	14/118	2/40	0.363
Donor gender	34/96	6/36	0.205
Pre-op diagnosis			0.05
Ischaemic heart disease	60 (46 %)	27 (64 %)	
Dilated cardiomyopathy	66 (50 %)	14 (33 %)	
Other	5 (4 %)	1 (2 %)	
Lipid-lowering drug therapy	35 (27 %)	20 (48 %)	0.018
Hypertension	87 (66 %)	33 (79 %)	0.122
Diabetes	3 (2 %)	1 (1 %)	1.0
Cytomegalovirus infection	42 (32 %)	20 (48 %)	0.063
Rejection episodes, median (range)	1 (0–12)	2 (0–8)	0.247
HLA-A-mismatch 0	5(4 %)	6 (15 %)	0.024
1	64 (52 %)	14 (34 %)	
2	55 (44 %)	21 (51 %)	
HLA-B mismatch 0	9 (7 %)	1 (2 %)	0.525
1	42 (34 %)	14 (34 %)	
2	73 (59 %)	26 (63 %)	
HLA-DR mismatch 0	11 (9 %)	4 (11 %)	0.440
1	65 (56 %)	17 (47 %)	
3	41 (35 %)	15 (42 %)	

The variables that were significantly different in the two groups on univariate analysis included time from transplantation, lipid-lowering therapy, a pre-operative diagnosis of ischaemic heart disease, Lp(a), total cholesterol, HDL cholesterol and triglycerides.

Although gender and hypertension were not significantly related to the presence of CAVD in this study, they were included in the multivariate analysis to avoid confounding. After adjusting for gender, hypertension, pre-operative diagnosis of ischaemic heart disease, use of lipid-lowering therapy and time from transplantation, Lp(a) remained significantly correlated with CAVD (likelihood ratio chi-squared = 3.93, $P = 0.05$). The results of the logistic regression analysis are summarised in Table 3. The addition of total cholesterol and HDL cholesterol or trigycerides alone to the above model indicated that each of these was a statistically significant independent risk factor while Lp(a) remained significant.

Table 2 Lipid and lipoprotein(a) levels in patients with and without cardiac allograft vascular disease (CAVD)

Variable – median (range)	No CAVD ($n = 132$)	CAVD ($n = 42$)	P
Apolipoprotein A1 (g/l)	1.3 (0.8–1.9)	1.3 (0.9–1.8)	0.332
Apolipoprotein B (g/l)	1.0 (0.3–1.7)	1.0 (0.5–2.0)	0.057
Total cholesterol (mmol/l)	6.3 (3.4–8.9)	6.8 (4.2–9.5)	0.021
High-density cholesterol (mmol/l)	0.9 (0.5–1.9)	0.8 (0.4–1.7)	0.002
Low-density cholesterol (mmol/l)	4.2 (2.0–6.5)	4.5 (2.5–7.2)	0.258
Triglycerides (mmol/l)	2.0 (0.5–6.7)	2.8 (0.7–8.3)	0.001
Lipoprotein(a) (mg/l)	95 (10–1875)	317.5 (10–2250)	0.031

Table 3 Results of multivariate logistic regression analysis (*Pre-op IHD*, pre-operative diagnosis of ischaemic heart disease)

Variable	Odds ratio	95 % Confidence intervals	Significance from Wald test
Gender	1.71	0.514, 2.668	0.706
Hypertension	1.516	0.605, 3.796	0.365
Pre-op IHD	1.099	0.445, 2.713	0.836
Time from transplantation (days)	1.015	1.002, 1.029	0.026
Lipid-lowering therapy	2.186	0.908, 5.259	0.076
Lipoprotein(a) (mgl/l)	1.318	1.004, 1.731	0.048
Triglycerides (mmol/l)	1.652	1.244, 2.195	0.001

Whilst there was a statistically significant difference between the two groups in the distribution of HLA mismatches at the A locus, it was not considered clinically significant as the association was not in a consistent direction. Indeed, the difference was not significant when a test recognising the ordinal nature of the number of mismatches was used (Mantel-Haenzsel test for linear association, $P = 0.73$).

Discussion

The role of immunological damage to the endothelium in the pathogenesis of CAVD has been the focus of much recent research [7]. Conventional risk factors including hyperlipidaemia may well contribute to the progression of the condition, regardless of the mechanism of the initial endothelial injury; hypercholesterolaemia has been shown to cause intimal thickening in the coronary arteries in a rabbit cardiac allograft model [1]. This large cross-sectional study confirms the association of hyperlipidaemia with CAVD seen by other groups [2, 8].

Lipoprotein antigen, or Lp(a), first described by Berg over 30 years ago [3], is a modified form of low-density lipoprotein (LDL). Each LDL molecule contains one molecule of apo B, and in what is usually a small proportion of circulating LDL, there is another protein, apolipoprotein (a), which has close structural homology with plasminogen [21]. LDL modified by apo(a) in this manner is known as Lp(a). Several case-control studies and one prospective study have demonstrated the association of Lp(a) with coronary artery disease [12, 19]. Lp(a) binds to plasminogen receptors in vitro [13] and it has been postulated that it may interfere with plasminogen activation, thus promoting thrombosis. Survivors of myocardial infarction who do not spontaneously recanalise the infarct artery have been shown to have higher Lp(a) concentrations than those with a patent artery [22]. An alternative hypothesis is that Lp(a) has a role in atherogenesis rather than thrombosis; there is now evidence of preferential retention of Lp(a) in atheromatous lesions via fibrin-binding in contrast to other apo-B-containing LDL [28].

The current study, involving a large number of cardiac transplant recipients, suggests that a high Lp(a) level is independently associated with CAVD. Because of pre-transplant pathology and drug therapy post-transplant, hyperlipidaemia is common in cardiac transplant recipients. The fibric acid derivatives, 3-methylglutaryl coenzyme reductase inhibitors and ion-exchange resins do not significantly lower Lp(a) concentrations [25]. A recent study also showed that body mass index, alcohol consumption and use of drugs for the treatment of diabetes and hypertension do not correlate with Lp(a) levels [15]. There have been conflicting reports on the effect of immunosuppressive therapy on Lp(a) levels. While Farmer et al. reported a decrease in Lp(a) levels with time after cardiac transplantation [9], Webb et al. found that there was an increase [31].

In contrast to other studies [14, 29], cytomegalovirus infection was not significantly associated with CAVD in our patients although there was a trend in this direction. Hypertension and HLA-DR mismatch, which have been associated with CAVD by other groups [6], were not significant risk factors in our study. A pre-operative diagnosis of ischaemic heart disease was associated with CAVD in our patients. While these patients might be expected to have higher lipid levels, the association with hyperlipidaemia remained significant after adjusting for the pre-operative diagnosis. While the use of steroids as part of the immunosuppressive regimen has been associated with higher lipid levels [18], there was no significant difference in the number of patients on steroids or the dose of prednisolone in patients with and without CAVD in our study.

We did not use quantitative coronary angiography or intravascular ultrasound in the diagnosis of coronary artery disease in this study. While we accept that using

these techniques may have increased the number of patients with coronary disease, we do not believe that it would have altered the main message of the study, i.e. the relationship between hyperlipidaemia [including high levels of lipoprotein(a)] and allograft vascular disease.

Until recently, there have been no studies demonstrating a beneficial effect of lipid-lowering therapy on the incidence of CAVD. A recent paper describing a beneficial effect of pravastatin on the incidence of acute rejection and on allograft vascular disease as assessed by intimal thickening measured with intravascular ultrasound [16] is an exciting development that needs to be duplicated at other centres. For now it would certainly appear reasonable to treat those patients who do not develop side effects with the drugs used. Most currently used drugs do not affect Lp(a) concentrations, and it is possible that when such therapy is available the impact on CAVD will be greater. It is likely that large multicentre studies will be required to demonstrate such an effect.

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