LIPOPROTEIN PROFILE IN DIALYSIS AND TRANSPLANT PATIENTS

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SYNOPSIS

The lipoprotein profile was assessed in three groups of renal patients. Patients with uraemia whether dialyzed or not, showed raised levels of serum triglyceride due to increase in triglyceride contents in the VLDL and LDL lipoprotein fractions, and significantly decreased HDL cholesterol. Transplant patients showed, besides hypertriglyceridaemia, a raised level of serum cholesterol and normal HDL cholesterol content.

The prevalence of lipid abnormalities in patients with chronic renal failure (not on dialysis), haemodialysis patients and transplanted patient was 29%, 50% and 63% respectively. Further analysis revealed no differences in serum insulin and serum PTH levels between subgroups of patients who were normolipidaemic or hyperlipidaemic. The pathogenesis of hyperlipidaemia remains unclear and its relationship to atherosclerotic deaths has still to be established.

INTRODUCTION

The concept that prolonged maintenance haemodialysis accelerate atherosclerosis was first advanced by Lindner et al in 1974 (1) when they observed that after more than 5 years of dialysis 8 out of 10 deaths were due to atherosclerotic complications. This hypothesis was supported by other studies (2, 3) which also noted that the majority of renal patients had several risk factors that are often implicated in the pathogenesis of atherosclerosis; risk factors such as hypertension, hyperlipidaemia, glucose intolerance, hyperuricaemia, hyperparathyroidism, cigarette smoking, obesity and a sedentary existence. Much research has been directed to elucidate the possible mechanism of atherosclerosis in renal patients particularly in the area of lipid metabolism (4, 5, 6) as hyperlipidaemia has been established as one of the major risk factors for cardiovascular diseases.

Between 1968 to 1980 there were 64 deaths among patients who were on haemodialysis in our department. Cardiovascular accidents had accounted for 17 deaths or 27%. The status with respect to risk from atherosclerotic complications has so far not been investigated in our renal patients. In this study, we measured the lipoprotein profile and other biochemical parameters in these patients and compared them to patients with chronic renal failure (not on dialysis) and transplant patients

METHODS

SUBJECTS

Patients with a history of diabetes mellitus, SLE, arthritis, the nephrotic syndrome and hyperuricaemia were excluded from this study. Forteen patients in renal failure (not on dialysis) (9 males, 5 females; age 35 +/− 11 years; serum creatinine 1584 +/− 690 umol/l; mean +/− SD) were studied 1 to 36 (mean 13) months after initial diagnosis. All but one had hypertension. Twenty dialysis patients (17 males, 3 females; age 35 +/− 7 years; serum creatinine 1071 +/− 257 umol/l; mean +/− SD) were studied 8 to 93 (mean 41) months after initiation of dialysis treatment (average 3 x 5 hours weekly). Again all except one had hypertension and were treated. Twenty-seven transplant patients (13 males, 14 females; age 29 +/− 8 years; serum creatinine 124 +/− 71 umol/l mean +/− SD) were studied 10 to 56 (mean 31) months after their renal transplantation. All these patients were on prednisolone and azathioprine immunosuppressive therapy. 19 in this group of 27 patients were treated for hypertension. For control values, blood was taken from 27 members of the staff (11 males, 16 females; age 34 +/− 9 years; serum creatinine 71 +/− 18 umol/l; mean +/− SD).

BIOCHEMICAL MEASUREMENTS

All patients and controls were fasted for 12 to 14 hours overnight before a 15 ml blood sample was taken. Dialysis patients were sampled just prior to a dialysis session. Fasting serum was observed for lactescence and refrigerated for 24 hours to screen for chylomicrons. Serum triglyceride and cholesterol were measured using enzymatic kits (Roche; CV for triglyceride 2%, CV for cholesterol 3%), insulin by RIA kits (Serono; CV 12%), PTH by RIA kits (Cambridge; CV 6%) and creatinine by a micromethod (7) using cation exchange resin to remove interfering substances (CV 4%).

LIPOPROTEIN SEPARATION

Lipoproteins were isolated and quantitated as described by Bagdade et al (4). Serum, 4 ml, was overlayered with 2.5 ml of 0.15M NaCl solution and centrifuged (Beckman L5-50) for 18 hours at 40,000 rpm in the 50.3Ti angle rotor at 10 degree Celcius to flotate all the very-low-density lipoproteins (VLDL) into the upper 2.5 ml of the tube. The top third of the tube was sliced off and the infranatant containing the low-density (LDL) and high-density lipoprotein (HDL) was collected quantitatively and made up to the original serum volume of 4 ml with 0.15M NaCl solution. Aliquots of this fraction and whole serum were analyzed for cholesterol and triglyceride contents. VLDL and LDL were precipitated by heparin-manganese as described by Burstein et al (8). To 0.5 ml of serum was added 0.02 ml of 5% heparin solution and 0.025 ml of 1 M manganese chloride. A precipitate appeared immediately and after thorough mixing, the mixture was centrifuged for 5 min at 14,000 g (Microcentrifuge). The clear supernatant was used for determination of HDL cholesterol and triglyceride. Cholesterol and triglyceride contents of the VLDL were calculated by subtracting the values obtained with the infranatant from the total serum values. And similarly, LDL cholesterol and triglyceride values were calculated by the differences obtained between the infranatant and the HDL values. Standard deviation and Student's t test were used for statistical analysis.

RESULTS

WHOLE SERUM LIPIDS

Compared with the control group, all 3 patients groups had significantly increased total triglyceride levels (Table 1). With regard to total cholesterol both the undialyzed and dialyzed groups had normal levels, but the transplant patients had significantly elevated levels. Table 2 shows that 7% or 2 out of 27 subjects in the control group had hyperlipidaemia. In contrast, the incidence of hyperlipidaemia was 29% in the undialyzed, 50% in the dialyzed and 63% in the transplant patient groups.

Table 1. Fasting levels of whole serum triglyceride and cholesterol in control subjects, undialyzed, dialyzed and transplant patients.

STUDY GROUP	TOTAL TRIGLYCERIDE	TOTAL CHOLESTEROL
n = 27		
CONTROL	1.01 +/− 0.35	4.48 +/− 0.65
n = 14		
UNDIALYZED	1.57 +/− 0.73	3.89 +/− 1.24
p	<0.01	NS
n = 20		
DIALYZED	1.92 +/− 0.70	4.61 +/− 1.22
p	<0.001	NS
n = 27		
TRANSPLANT	2.06 +/− 1.25	5.67 +/− 1.04
p	<0.001	<0.001

All results are expressed in mmol/l (mean +/− SD)

Table 2 Prevalence of lipid abnormalities

STUDY GROUP	Number of patients with lipid abnormalities*			
	elevated TG	elevated CHOL	elevated TG & CHOL	TOTAL
n = 27 CONTROL	2	0	0	2 (7%)
n = 14 UNDIALYZED	3	0	1	4 (29%)
n = 20 DIALYZED	6	0	4	10 (50%)
n = 27 TRANSPLANT	5	4	8	17 (63%)

* having values greater than control mean plus 2SD

LIPOPROTEIN PROFILE

The undialyzed and dialyzed groups showed normal levels of LDL cholesterol but VLDL cholesterol was elevated in both groups (Table 3). However of greater importance is the reduced levels of HDL cholesterol in these two patient groups. In contrast, raised total serum cholesterol in the transplant group was reflected in a significant increase in LDL cholesterol. VLDL cholesterol was also increased. Significantly, the HDL cholesterol was not reduced but was slightly higher than normal.

The lipoprotein profiles as assessed by triglyceride contents was similar in all three groups of patients (Table 4). All 3 groups had hypertriglyceridaemia. Increased total triglyceride was mainly due to increased triglyceride contents in VLDL and LDL fractions in all 3 groups. HDL triglyceride which was normal in undialyzed and dialyzed patients was high only in transplant patients.

INSULIN AND PTH LEVELS

Serum insulin and serum parathyroid hormone (PTH) levels were measured in some of the patients (Table 5). Results were analyzed with hyperlipidaemic and normalipidaemic patients in each group treated as separate subgroups. Insulin level was normal in undialyzed patients. In contrast it was significantly raised in dialyzed and transplant patients and the high level of insulin occurred in both normolipidaemic and hyperlipidaemic subgroups. Serum PTH was significantly increased only in the dialyzed group. Here again both subgroups (normolipidaemic and hyperlipidaemic) showed high values. There was no difference between the subgroups with respect to age and number of months on treatment.

Table 3. Lipoprotein profiles based on lipoprotein cholesterol contest

STUDY GROUP	VLDL	LDL	HDL
n = 27 CONTROL	0.34 +/− 0.23	3.13 +/− 0.62	1.01 +/− 0.31
n = 14 UNDIALYZED	0.57 +/− 0.54	2.67 +/− 0.91	0.67 +/− 0.28
p	NS	NS	<0.01
n = 20 DIALYZED	0.73 +/− 0.36	3.13 +/− 1.14	0.78 +/− 0.31
p	<0.001	NS	<0.005
n = 27 TRANSPLANT	0.80 +/− 0.62	3.81 +/− 0.88	1.09 +/− 0.28
p	<0.01	<0.001	NS

All results are expressed as cholesterol in mmol/1 (mean +/− SD)

DISCUSSION

The mean triglyceride level was significantly higher in 14 undialyzed and 20 dialyzed uraemia patients studied when compared to a group of 27 control subjects. The hypertriglyceridaemia was reflected in the increased triglyceride contents of VLDL and LDL lipoproteins. Total serum cholesterol was normal in these two groups of uraemic patients and this is again confirmed by the normal LDL cholesterol levels. However of greater significance is the mean HDL cholesterol values which were significantly lower in the undialyzed and dialyzed patients when compared with normal subjects. This is important because low HDL cholesterol has been established as the most important single indicator of increase risk from atherosclerotic disease (9). Our findings are in accord with other similar studies (3, 4, 6). Patients after transplantation showed a different lipoprotein profile. Both the mean serum triglyceride and serum cholesterol levels were raised significantly above that of the control group with concomitant increases in cholesterol and triglyceride contents in VLDL and LDL fractions. The HDL cholesterol level on the other hand was normal. This is in contrast to the finding of Bagdade et al 1977 (10), but others (6, 11) had noted that plasma HDL cholesterol in transplant patients often return to normal or near normal.

Bagdade et al (12) found hyperinsulinaemia in uraemia patients and suggested its contribution to triglyceride elevation in uraemia by increasing hepatic synthesis. A role for the parathyroids was also indicated when Lindall et al (13) measured significant reduction in insulin hypersecretion following parathyroidectomy in dialyzed patients. We had measured serum insulin and parathyroid hormone in some of our patients. Serum insulin was found raised in both the dialyzed and transplant patients whereas PTH was raised only in the transplant group. However, high levels were found in both hyperlipidaemic and normolipidaemic patients when they were analyzed as separate subgroups. It would appear that if hyperinsulinism and hyperparathyroidism were contributing factors to the hyperlipidaemia in renal patients, they are probably only of secondary importance.

There is no doubt that there are lipid imbalances in both uraemic and transplant patients. In the patients with chronic renal failure, whether dialyzed or not, these are attributed to the uraemic state and the retention of uraemic toxins that inhibit enzymes in various metabolic pathways (6). In the transplant patients where the uraemic condition had been corrected, these are related to the steroid therapy (6). in this connection, patients on alternate day steroid therapy have been reported to have improved lipid metabolism (5). Whether these lipid abnormalities are the significant causes of 'accelerated atherosclerosis' in dialysis and transplant patients is still not clear. In fact this concept has been questioned. It was claimed that when patients with a history of atherosclerotic complications were excluded (14) and when only cardiovascular deaths with an atherosclerotic pathology were included (15), then accelerated atherosclerosis was not found to be a major problem among the renal patients. An important study on this question had recently been reported (16) where the authors analyzed mortality risk factors in 1453 patients treated by chronic haemodialysis. They noted a high incidence of cardiovascular deaths (43%) which however bore no correlation with the high body mass index, elevated cholesterol, triglyceride and uric acid, but was associated with a poor nutritional state and/or low protein intake and elevated blood pressure.

In conclusion, significant hyperlipidaemia is found among uraemic patients. This lipid disturbance is not corrected by dialysis or even after successful transplantation. The pathogeneis is still not clear and its contribution to atherosclerotic complications in renal patients remains to be established.

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Table 4. Lipoprotein profiles based on lipoprotein triglyceride contents

STUDY GROUP	VLDL	LDL	HDL
n = 27			
CONTROL	0.55 +/− 0.32	0.27 +/− 0.07	0.21 +/− 0.07
n = 14			
UNDIALYZED	0.88 +/− 0.63	0.49 +/− 0.14	0.21 +/− 0.06
p	<0.05	<0.001	NS
n = 20			
DIALYZED	1.16 +/− 0.57	0.52 +/− 0.16	0.23 +/− 0.08
p	<0.001	<0.001	NS
n = 27			
TRANSPLANT	1.32 +/− 1.21	0.43 +/− 0.16	0.31 +/− 0.10
p	<0.01	<0.001	<0.001

All results are expressed as triglyceride in mmol/1 (mean +/− SD)

Table 5. Comparison of various clinical and biochemical parameters between patients with and without hyperlipidaemia in each patient group.

STUDY GROUP	INSULIN	PTH	AGE	TREATMENT
SUBGROUP	mU/1	pg/ml	years	months
n	23	13	27	
CONTROL	13.5 +/− 3.5	397 +/− 35	34 +/− 9	—
UNDIALYZED				
n	6	6	10	10
Normolipidaemic	15.5 +/− 4.1	426 +/− 94	34 +/− 11	13 +/− 12
p	NS	NS	NS	
n	4	4	4	3
Hyperlipidaemic	13.0 +/− 2.6	412 +/− 82	37 +/− 10	12 +/− 12
p	NS	NS	NS	NS
DIALYZED				
n	8	4	10	10
Normolipidaemic	23.1 +/− 7.0	584 +/− 215	34 +/− 8	43 +/− 27
p	<0.001	< 0.01	NS	
n	9	6	10	10
Hyperlipidaemic	18.3 +/− 3.0	501 +/− 37	36 +/− 5	39 +/− 18
p	<0.01	<0.001	NS	NS
TRANSPLANT				
n	10	3	10	10
Normolipidaemic	20.3 +/− 3.5	344 +/− 61	30 +/− 7	32 +/− 15
p	<0.001	NS	NS	
n	17	11	17	17
Hyperlipidaemic	22.9 +/− 6.7	473 +/− 150	29 +/− 9	30 +/− 14
p	<0.001	NS	NS	NS

All results are expressed as mean +/− SD