Original article	Protease inhibitor related type III hyperlipoproteinaemia is common and not associated with apolipoprotein-E E2/E2 phenotype

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Objective: To determine the prevalence of type III hyperlipoproteinaemia in a cohort of HIV infected patients taking protease inhibitors and its correlation with the apolipoprotein-E2 isoform.

Design: Cross sectional study of 57 consecutive HIV infected subjects taking protease inhibitor therapy for a median of 12.5 (1–29) months, seen in an outpatient HIV clinic. Controls were 17 patients on non-nucleoside reverse transcriptor inhibitor therapy (NNRTI) for 9 (1–19) months and 50 antiviral naive patients.

Methods: Fasting cholesterol, triglyceride, HDL cholesterol, lipoprotein (a), and glucose were measured. Lipoprotein electrophoresis was performed on patients with a cholesterol >6.5 mmol/l and a triglyceride concentration of >4.5 mmol/l. Apolipoprotein-E phenotype was determined in serum.

Results: Dyslipidaemia was found in 43 (75%) PI treated patients—37 with triglyceride >2.3 mmol/l, 30 with cholesterol >6.5 mmol/l, and nine with HDL cholesterol <0.9 mmol/l. 38% had a lipoprotein (a) >300 mg/l. 11 patients (19.3%) had a type III hyperlipoproteinaemia pattern. Only one was homozygous for the E2 phenotype and none had clinical diabetes. An additional patient had a serum lipid profile compatible with type III hyperlipoproteinaemia and an E3/E2 phenotype in whom electrophoresis was not carried out before treatment. Six (35%) of the NNRTI and 16 (32%) of the antiviral naive patients had dyslipidaemia. 18 (31.6%) of the PI and none of the control patients had a cholesterol and/or triglyceride >8 mmol/l.

Conclusion: Type III hyperlipoproteinaemia is common in this group of patients and need not be associated with the apolipoprotein-E2/E2 isoform. HIV protease inhibitors may interfere with lipoprotein receptor related protein.

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Introduction

The use of protease inhibitors (PI) in the treatment of HIV positive patients has been associated with a syndrome consisting of abnormal body fat distribution, hyperlipidaemia, impaired glucose tolerance, and insulin resistance.[1–7] The most common lipid abnormality is raised serum triglyceride concentrations. However, increased total cholesterol and reduced HDL cholesterol concentrations have been shown[1 5 6] and increases in serum lipoprotein(a) [Lp(a)] levels[6 8] are also reported. Behrens *et al* have reported that the commonest lipoprotein abnormality was Fredrickson type IV (37%) followed by IIb (36%) and IIa (18%) and V (7%).[8] None of their cohort had Fredrickson type III hyperlipoproteinaemia.

Lister *et al*[9] have recently reported a patient with latent type III hyperlipoproteinaemia precipitated by PI therapy. This patient had the E2/E2 isoform for apolipoprotein E which is seen in 95% of patients with this form of dyslipidaemia.[10]

We studied a group of HIV infected patients taking protease inhibitors to assess their lipid profile. We measured their fasting serum cholesterol, triglyceride, HDL cholesterol, and Lp(a). Where patients had a raised cholesterol and a triglyceride concentration >4.5 mmol/l lipoprotein electrophoresis was performed and apolipoprotein E phenotype determined.

Patients and methods

In a cross sectional study 57 consecutive patients receiving PI for a median of 12.5 (1–29) months were investigated. The patient group consisted of 48 men, of whom 44 were homosexual, four were black (black African, black African Caribbean, and black other), two were of Asian origin, and none were injecting drug users. The PI use in the patients was as follows; 26 were taking ritonavir alone, seven were taking a ritonavir/saquinavir combination, four were taking indinavir, and 20 were taking nelfinavir. Thirty (53%) were antiretroviral naive before starting PI. For comparison we used 17 consecutive patients taking a non-nucleoside reverse transcription inhibitor (NNRTI) for a median period of 9 months (range 1–19). All but one was naive to therapy before starting NNRTI. Details of the two patient groups including nucleoside analogues they had taken for >1 month at any time is given in table 1. Controls were 50 HIV positive patients naive to antiretroviral therapy before they went on to highly active antiretroviral

Table 1 Demographic, drug history, and number with abnormal lipids in patients taking protease inhibitors (PI), non-nucleoside reverse transcription inhibitors (NNRTI), and naive to antiretroviral therapy. Details of nucleoside analogues (NRTI) taken by the patient at any time in their life for >1 month are also given

	PI (n=57)	NNRTI (n=17)	Naive (n=50)
Median age (range)	40 (23–56)	38 (19–66)	38 (19–65)
White	51	11	40
Women	9	3	9
Homosexual	44	9	34
Months on HAART (range)	12.5 (1–29)	9 (1–19)	—
NRTI			
AZT	51	15	
D4T	38	4	
3TC	48	15	
ddI	34	3	
ddC		1	
Dyslipidaemia (number)			
Cholesterol >6.5 mmol/l	30	1	1
HDL cholesterol <0.9 mmol/l	9	4	17
Triglyceride >2.3 mmol/l	37	3	10
Lipoprotein(a) >300 mg/l	19/50*	7	8/24*

HAART = patients taking three or more antiviral drugs.
*Not all patients had lipoprotein(a) measurement.

therapy (HAART) therapy (table 1). Forty had later lipid measurement on HAART (27 on PI and 13 on NNRTI). The controls are part of an ongoing prospective study of metabolic changes in HIV infected subjects on PI and NNRTI. The study has obtained approval from the local ethics committee. Clinical diabetes was present at the time samples were taken in three of PI, and one each of NNRTI and antiretroviral naive patients. Lipodystrophy was diagnosed from the patient's history of change in body habitus and on clinical examination. No formal tests of fat distribution were made. Blood was obtained after a 12 hour fast. Total cholesterol triglyceride, HDL cholesterol, and glucose were measured by standard enzymatic laboratory methods (Biostat Diagnostics Cheshire UK (Lipids and HDL-c); Instrumentation Laboratory, Warrington, UK (Glucose)). Lipoprotein(a) was measured by immunoturbidity (Diasorin, Wokingham, UK) and lymphocyte phenotype, HIV-RNA viral load (Roche Amplicor) were measured by standard laboratory methods at the clinical chemistry laboratory. In addition, in all but two patients who had triglyceride >4.5 mmol/l and cholesterol >6.5 mmol/l concentrations, lipoprotein electrophoresis on agarose (Sebia Analytical Technologies, Farnborough, UK) was performed. In these subjects apolipoprotein-E phenotyping was carried out using isoelectric focusing followed by western blotting using an

adaptation of the method of Havekes *et al.*[11] For the purpose of this study dyslipidaemia was defined as one or more of the following: a total cholesterol of >6.5 mmol/l, HDL cholesterol of <0.9 mmol/l, triglyceride of >2.3 mmol/l. A lipoprotein(a) >300 mg/l was considered abnormal but was not included in the definition of dyslipidaemia.

Results

Dyslipidaemia was found in 43 (75%) patients on PI: 37 with triglyceride >2.3 mmol/l, 30 with cholesterol >6.5 mmol/l, and nine with HDL cholesterol <0.9 mmol/l, of whom 14 also had clinically apparent lipodystrophy (table 1). They had been exposed to antiretroviral therapy for a median of 25.5 (range 4–61) months and the median duration of PI therapy was 11 (1–24) months. Fourteen patients did not have dyslipidaemia, one of who had lipodystrophy. Median exposure to antiretroviral and PI therapy for the patients without dyslipidaemia was 8 (1- 42) and 7.5 (1–29) months, respectively. Eighteen (31.6%) of the patients exposed to PI therapy had moderate to severe dyslipidaemia (defined as fasting cholesterol and/or triglyceride >8 mmol/l).

Twelve patients had a lipid profile that was typical of type III hyperlipoproteinaemia. Electrophoresis of 11 of these subjects was undertaken (table 2). Electrophoresis showed that nine of these subjects had a broad β band with no obvious VLDL and LDL bands, and two patients (AB, CW) had a broad β band with very faint LDL and VLDL bands. These data are consistent with type III hyperlipoproteinaemia. One was homozygous for the E2 phenotype. In one additional female subject (JE, table 2) there was near equimolar increase in cholesterol and triglyceride but electrophoresis of this sample was not undertaken before therapy. This patient had an E3/E2 phenotype. Five other patients with moderate to severe dyslipidaemia had an electrophoresis pattern showing increased VLDL bands, three with an associated increased LDL band. None had clinical diabetes. One patient (GR) had a 2 hour blood glucose level of 9.6 mmol/l after a standard 75 g load of glucose. Seven of the 11 patients had lipodystrophy.

Dyslipidaemia, as defined above, was seen in six (35%) of subjects on NNRTI including the patient with diabetes (table 1). One had a

Table 2 Fasting lipid profile and apolipoprotein-E phenotype in HIV infected patients with type III dyslipidaemia taking protease inhibitors

Patient	Age	Sex	PI	Time on PI (weeks)	Total cholesterol (mmol/l)	HDL cholesterol (mmol/l)	Triglyceride (mmol/l)	Lipoprotein-(a) (mg/l)	Phenotype	Lipodystrophy
AB	33	M	I	60	7.1	0.8	9.1	747	E2/E2	Yes
PC	37	M	N	13	11	0.4	23	236	E3/E3	No
JE*	37	F	R	5	11	0.8	12	306	E3/E2	Yes
RF	50	M	I	16	15.2	0.7	27	62	E3/E3	Yes
CF	40	M	R	25	8.2	0.8	7.9	192	E4/E3	Yes
JG	31	M	R	17	9.3	0.7	9.6	237	E4/E3	No
ML	31	M	R	24	10.2	0.9	5.4	147	E3/E3	No
TP	39	M	N†	76	6.8	0.8	7.8	184	E4/E2	Yes
GR	44	M	R/S	26	8.7	0.5	12	192	E3/E3	Yes
AR	34	M	R	25	8.9	0.9	11	417	E3/E3	No
CW	43	M	R/S	29	8.4	1	10.6	198	E3/E3	Yes
MW	50	M	R	26	13.2	0.8	18	501	E3/E2	Yes

I = indinavir, N = nelfinavir, R = ritonavir, S = saquinavir.
*Electrophoresis was not carried out before therapy.
†Had ritonavir for 7 and nelfinavir for 12 months.

serum cholesterol of 7.3 mmol/l and a serum triglyceride of 5.6 mmol/l, but his weekly alcohol intake was 40–60 units. His lipoprotein electrophoresis showed dense pre-β and β bands, with a diffuse stain of lipid which may represent IDL. Twenty one (42%) retroviral naive patients had dyslipidaemia, only two with triglyceride > 4.5 mmol/l. One was diabetic, and another with a triglyceride level of 7.3 mmol/l subsequently developed type III dyslipidaemia on antiviral therapy (table 2, patient AB). None of the NNRTI or naive patients had lipodystrophy. Lipoprotein(a) >300 mg/l was observed in 19 of 50 (38%) receiving PI, seven (41%) receiving NNRTI, and 33% of retroviral naive patients in whom it was measured (table 1).

Discussion

Abnormalities in lipid metabolism are common in patients receiving PI.[1–6] Hypertriglyceridaemia can also occur in HIV positive patients not on antiviral therapy[12] and there is evidence of increased hepatic lipogenesis in HIV.[13] Other metabolic abnormalities reported in patients on antiretroviral therapy include lactic acidosis and abnormalities in adrenal steroids.[14 15] In our study, 75% of the patients exposed to PI therapy had one or more abnormalities in their serum lipids and nearly 32% of the patients had moderate to severe dyslipidaemia. Type III hyperlipidaemia was common and accounted for 19.3% of the subjects. None of them had clinical diabetes. Dyslipidaemia was also seen in 35% of patients receiving NNRTI and 42% of treatment naive individuals. Two of the three patients with moderate to severe abnormality had diabetes and an E2/E2 phenotype respectively.

In the only other study that reported lipoprotein electrophoresis in 38 patients treated with PI,[8] type III hyperlipoproteinaemia was not found. In a recent report, however, Sosmann *et al* showed that serum LDL and IDL were raised in patients taking a PI containing regimen compared with a matched group on PI sparing regimens.[16] This latter abnormality is associated with "broad band" or intermediate density lipoprotein (IDL) which can be specifically demonstrated using lipoprotein electrophoresis. Cholesterol and triglyceride concentrations are usually equimolar. Type III hyperlipoproteinaemia is characterised by increased cholesterol and triglyceride concentrations as a result of the accumulation of VLDL remnants and IDL. Over 95% of the patients with type III hyperlipoproteinaemia are found to have the E2/E2 phenotype and it may be associated with a variety of clinical disorders including diabetes, growth hormone or pituitary deficiency, thyroid disease, or myeloma. ApoE is the ligand for at least two lipoprotein receptors—the LDL (or apo B/E) receptor and the putative hepatic apoE receptor (or LDL receptor related protein, LRP). Amino acid residues 140–160 of the apoE molecule contain a high affinity LDL receptor binding site, and surrounding residues may be responsible for the proper alignment of the binding site and so affect receptor binding.

Loss of a positive charge at amino acid residues 158 or 112 associated with a change in amino acid from arginine to cysteine are the basis of the decrease in apoE2 binding and the accumulation of VLDL remnants and IDL.

Type III hyperlipoproteinaemia is encountered with a frequency of <1:10 000 in the population.[10] Our results suggest that this condition is not uncommon in PI treated HIV infected patients and need not be typically associated with the E2/E2 phenotype. It is possible that HIV protease inhibitor, which shares sequence homology with lipoprotein receptor related protein,[17] might itself interfere with the removal of intermediate lipoprotein from the circulation. We believe this finding may help in the understanding of HIV associated dyslipidaemia. Studies in normal subjects taking ritonavir for 2 weeks showed that, while cholesterol and triglyceride increased in IDL particles, most of the triglyceride enrichment occurred in the VLDL fraction.[18] However, it is possible that a longer duration of therapy might be required for the IDL accumulation to become apparent. Our study only examined patients with lipoprotein electrophoresis when dyslipidaemia, as defined above, was apparent. We note with interest that two patients had an electrophoresis of their serum showing increases in the VLDL and LDL factions which preceded subsequent demonstration of a broad β band typical of type III hyperlipoproteinaemia. As the presence of a broad β band is open to some degree of subjective interpretation, a more definitive method for diagnosing type III hyperlipoproteinaemia would best be done by ultracentrifugation to identify the β migrating lipoproteins in density <1.006 g/ml.[19] However, our results are compatible with a recent report by Bonnet *et al*[20] who showed a 2–3-fold increase in apo-E levels in HIV infected patients taking a PI.

Although Lister *et al*[9] reported an apolipoprotein E2/E2 phenotype in a patient presenting with type III hyperlipoproteinaemia, our study shows most patients with serum electrophoretic pattern compatible with type III hyperlipoproteinaemia do not exhibit this apolipoprotein-E phenotype. Type III hyperlipoproteinaemia is associated with an increased risk of atherosclerosis.[6] Moreover, raised triglyceride concentrations have been shown to be a statistically significant relative risk estimate of coronary heart disease, even adjusting for HDL in subjects with a total cholesterol/HDL cholesterol ratio >5.[21] An increased risk is also associated with an abnormal lipoprotein(a) level[22] and central obesity. Assessing risk for coronary heart disease is best based on guidelines[23] though long term benefit has not been shown in this group of patients. In the absence of such data it would seem prudent to try changing to a PI sparing regimen which may[24] or may not be effective,[25] or to treat with lipid lowering drugs.[26] Hypertriglyceridaemia >11 mmol/l should be treated to prevent the potential problem of pancreatitis.

and read the electrophoresis; MC performed the phenotyping; HJ and YDeS collected the data; MS wrote manuscript with input from all authors.

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