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Lipid profile in adult patients with Fabry disease - Ten-year follow up

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ABSTRACT

Background: Fabry disease, an X-linked genetic condition, results from alpha-galactosidase deficiency and increased accumulation of glycosphingolipids in cardiovascular tissues. Clinical manifestation includes vasculature associated complications. Hyperlipidaemia is one of the cardiovascular risk factors however it has never been well defined in Fabry disease. Enzyme Replacement Therapy (ERT) is available but its effect on serum cholesterol is unknown. The aim of this project was to assess the influence of long-term ERT on lipid profile in a large cohort of adult patients with Fabry disease.

Methods: This was a retrospective analysis of lipid profile results. Patients with Fabry disease were on ERT for 10 years, were not treated with statins and had no severe renal impairment. All patients had lipid profile measured before ERT was commenced and 6, 12, 24, 36, 48, 60, 120 months later. Statistical analysis included ANOVA, Student *t*-test and descriptive statistics.

Results: Among 72 patients, 40 were females (median age 45; range 29–75), 32 males (median age 46; range 20–69). There was no significant difference in total cholesterol or HDL-cholesterol measured at baseline before ERT was commenced and 6, 12, 24, 36, 48, 60 and 120 months after ERT was commenced in 72 patients (ANOVA; $P = 0.673$ and $P = 0.883$, respectively). Female patients on ERT had higher mean HDL-cholesterol as compared to female patients with Fabry disease who were asymptomatic and not treated ($P \geq 0.05$). Total cholesterol between treated and non-treated female patients was comparable. Female patients on ERT have higher total cholesterol and HDL-cholesterol when compared to lipid results in male patients on ERT. Total cholesterol/HDL-cholesterol ratio was low in female and male patients on ERT over 10 years.

Conclusion: Adult patients with Fabry disease have remarkably elevated HDL-cholesterol and as a result, elevated total cholesterol. It is possible that elevated HDL-cholesterol has a cardioprotective effect in patients with this condition. Long term ERT does not have a significant impact on lipid profile in female and male population with Fabry disease.

1. Introduction

Fabry disease (OMIM 301500), an X-linked genetic condition caused by alpha-galactosidase (EC 3.2.1.22) deficiency, is associated with increased accumulation of glycosphingolipids in cardiovascular tissues and leads to organ failure and premature death [1]. Affected male patients display clinical features of the disease but female carriers manifest with symptoms later in their life. The clinical manifestations consist of vasculature associated complications, but the pathophysiology is unclear. It was shown that the Fabry disease specific vascular lesions occur as a result of vascular dysfunction with major components being endothelial dysfunction, alterations in cerebral perfusion and athero-thrombogenesis [2,3]. Although some patients with Fabry disease may suffer from stroke by involvement of larger arteries, small-vessel disease causes cerebral complications and probably contributes to complications of the kidney and the heart [4,5,6].

Undoubtedly, other cardiovascular risk factors contribute to enhanced worsening of arterial performance. Hypercholesterolaemia with a markedly raised HDL-cholesterol was observed in patients with Fabry disease [7] and was previously associated with the occurrence of cardiovascular disease [8]. Atherosclerosis was previously described in several case studies [9,10,11].

Two forms of Enzyme Replacement Therapy (ERT) are available that may arrest the disease progression and reverse symptoms [12,13]. Treatment with ERT does not prevent the occurrence of new complications, although it is possible that earlier intervention may be more beneficial in this respect. It was demonstrated in a small study that ERT does not have a significant impact on total cholesterol, HDL-cholesterol,

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LDL-cholesterol and triglycerides [7]. Therefore, the aim of this project was to assess the influence of long-term ERT on lipid profile in a large cohort of adult patients with Fabry disease.

2. Methods

2.1. Study design and ethical consideration

This was a retrospective analysis of lipid profile results. All patients have their lipid profile requested as part of their routine care when attend our Metabolic Clinic appointments every 6 months. Data is documented in their Electronic Patient Record and was reviewed as part of a wider audit project to review practise and the benefit of routinely requested biochemical tests.

2.2. Patients

All patients included in the study had confirmed diagnosis of Fabry disease and were treated with Enzyme Replacement Therapy (alpha-galactosidase 1 mg/kg fortnightly) for 5–10 years. Entry criteria included: no statin therapy, eGFR > 60 mL/min and no proteinuria. The incidence of cardiovascular events, diabetes mellitus history and smoking history were reviewed. All patients had lipid profile measured before ERT was commenced and 6, 12, 24, 36, 48, 60, 120 months later.

2.3. Biochemistry tests

Serum lipid profile, including total cholesterol, HDL-cholesterol and triglycerides, was analysed using enzymatic method on Siemens Advia 2400 automated analyser in Clinical Biochemistry Department and expressed in mmol/L. LDL-cholesterol was calculated using Friedwald equation. Total cholesterol/HDL-cholesterol was automatically calculated.

2.4. Statistical analysis

Descriptive statistics: mean ± SD and median (minimum-maximum), were used to describe continuous variables. Percentages were calculated for categorical variables.

For normally distributed variables repeated measures, analysis of variance (ANOVA) was performed for testing the significance of the main effect of therapy (pre- vs. post-measures). The overall change in total cholesterol and HDL-cholesterol was calculated using ANOVA. The test was used to determine whether there was any significant difference between the means of independent groups of variables all together.

Changes in total cholesterol and HDL-cholesterol between male and female patients were analysed using two-tailed paired *t*-test and presented as means ± SD. Statistical tests were conducted using Stats Direct statistical software. *P*-value ≤ 0.05 was considered statistically significant.

3. Results

From 120 adult patients with Fabry disease, 72 met entry criteria and were included in the study (40 (56%) females, 32 (44%) males). Median age of female patients was 45 (29–75) and of male patients was 46 (20–69). Among female patients, 23 were treated with ERT for up to 10 years and 17 were asymptomatic and not treated. All male patients were treated with ERT.

Their lipid profile included complete sets of total cholesterol and HDL-cholesterol results at 8 different time points. LDL-cholesterol and triglycerides were not measured in all patients and results were missing.

3.1. Clinical presentation

Among females, none of patients had documented previous cardiovascular event i.e. stroke-like episode, 8 had arrhythmia, including 3 had pacemaker/defibrillator fitted. One had new onset diabetes mellitus. Among males, one patient had past medical history of cardiovascular event, 12 had arrhythmia, 4 had pacemaker/defibrillator inserted. No patients were treated with statins or had significant renal impairment. Patients were non-smokers or ex-smokers.

3.2. Genotype

In the female group: six patients had mutations N215S, five P259R, two Q119X, two G183S. The remaining mutations were: PE7X, C52G, G361R, R301Q, R342X, A257P, c.1184INSTAG, c.194 + 1G.A, c.677DEL G, c.695 T > C, c.7161NST, IVS2 + 4DELAG, IVS6-1G.A, P.6183S, P.A31V, P.G271D, P.T4101, P·W277C/P, T412 N, W287X.

In the male group, two patients had mutations A257P, seven N215S, two R112C, two R301Q and the remaining mutations were: P259R, Q119X, R227X, P293S, P342P, P.6183S, P.A31V, T141I, T412 N, Y88D, c.1011-1029DEL19, c.194 + 1G > A, c.350 T > G, c.793C > T, c.350 T > G, IVS2 + 4DELAG, IVS5-2DEL2BP.

3.3. Total cholesterol and HDL-cholesterol before and after ERT

There was no significant difference in total cholesterol or HDL-cholesterol measured at baseline and 6, 12, 24, 36, 48, 60 and 120 months after ERT was commenced in 72 patients (ANOVA; $P = 0.673$ and $P = 0.883$, respectively).

3.4. Total cholesterol and HDL-cholesterol in female patients on ERT vs no ERT

Overall, female patients on ERT had higher mean HDL-cholesterol as compared to female patients with Fabry disease who were asymptomatic ($P \geq 0.05$, Table 1B). Total cholesterol between treated and non-treated female patients was comparable (Table 1A).

3.5. Total cholesterol and HDL-cholesterol in female and male patients on ERT

Female patients on ERT have slightly higher mean total cholesterol and HDL-cholesterol compared to male patients on ERT (no statistical significance; $P \geq 0.05$, Table 2A and 2B).

Table 1A
Female patients on ERT vs non ERT; total cholesterol at baseline and at 6, 12, 24, 36, 48, 60 and 120 months of ERT. Normal cut off value for total cholesterol is < 5 mmol/L.

Mean total cholesterol (± SD)	Baseline	6 months	12 months	24 months	36 months	48 months	60 months	120 months
Females no ERT ($n = 17$)	5.1 ± 1.3	5.7 ± 1.4	4.9 ± 0.8	4.97 ± 0.9	5.38 ± 1.3	5.18 ± 1.2	5.2 ± 1	5.4 ± 1
Females ERT ($n = 23$)	5.16 ± 0.7	4.9 ± 0.5	5.4 ± 1	5.1 ± 1.16	5.11 ± 1	5.12 ± 0.97	5.32 ± 1	5.33 ± 1.2

Table 1B
Female patients on ERT vs non ERT; HDL-cholesterol at baseline and at 6, 12, 24, 36, 48, 60 and 120 months of ERT. Normal HDL-cholesterol cut off is > 1.1 mmol/L for men, > 1.2 mmol/L for females.

Mean HDL-cholesterol (± SD)	Baseline	6 months	12 months	24 months	36 months	48 months	60 months	120 months
Females No ERT (n = 17)	1.46 ± 0.4	1.6 ± 0.55	1.4 ± 0.24	1.4 ± 0.36	1.43 ± 0.5	1.5 ± 0.4	1.5 ± 0.4	1.6 ± 0.4
Females ERT (n = 23)	1.76 ± 0.5	1.6 ± 0.1	1.8 ± 0.5	1.8 ± 0.46	1.84 ± 0.4	1.9 ± 0.5	1.9 ± 0.5	1.8 ± 0.5

3.6. Total cholesterol/HDL-cholesterol ratio in female and male patients on ERT

Total cholesterol/HDL-cholesterol ratio was low both in female and male patients on ERT over 10 years and varied between 2.9 and 3.35 for female patients and between 2.9 and 3.15 for male patients.

4. Discussion

This is the largest set of lipid profile results for adult patients with Fabry disease who were treated with ERT over a period of 10 years. Whereas abnormally low levels of high-density lipoprotein (HDL), in association with severe coronary artery disease and myocardial infarction, have been previously observed in Fabry disease [10], we demonstrated that adult patients with Fabry disease have remarkably elevated HDL-cholesterol and as a result, total cholesterol that, in addition, were not affected by ERT. We observed that female patients on ERT had slightly higher, but not statistically significant, total cholesterol and HDL-cholesterol as compared to male patients on treatment and it is not clear if other hormonal factors or carrier status for Fabry disease affected their lipid metabolism.

The pathophysiology of lipoproteins in Fabry disease is thought to be affected by cellular glycosphingolipids accumulation and subsequent inhibition of apoA-I-mediated cholesterol efflux [14,15]. The cellular defect in cholesterol trafficking results from substrate accumulation and thereby suggests that the vascular disease observed in Fabry patients is caused by an alteration in the lipid homeostasis of the endothelial cells, accumulation of substrate in the endothelial cells [16] and upregulation of proinflammatory endothelial markers [17]. As a consequence, the arteriosclerosis in patients with Fabry disease is probably due to damage to vessel walls occurring as a result of defective glycosphingolipids metabolism and accumulation of glycosphingolipids in the tissue, rather than to abnormal low density lipoprotein (LDL) metabolism [17].

Additionally, low density lipoprotein (LDL) and high density lipoproteins (HDL) transport glycosphingolipids [18,19,20] from circulation to vascular cells through the low-density lipoprotein receptor that in turn leads to glycosphingolipids accumulation [21,22]. The defect in glycosphingolipids metabolism caused by the enzyme deficiency present in Fabry disease causes an even distribution of excessive plasma glycosphingolipids among several lipoproteins [23]. Therefore, ERT would be expected to correct this excess delivery of glycosphingolipids and their accumulation within endothelial cells. However, it has been shown that ERT has limited access to cells other than vascular endothelial cells [10]. The results of our study showed that ERT did not have an impact on lipoprotein concentration and it was in keeping with previous findings by Cartwright et al. (2004). It remains unanswered if ERT affects the binding between glycosphingolipids and lipoproteins.

As per the Joint British Societies' guidelines, the total cholesterol/HDL-cholesterol ratio above 6 is considered as high risk [25]. We demonstrated that in patients with Fabry disease on ERT, mean total cholesterol to HDL-cholesterol ratio was half of the target value indicating the low cardiovascular risk. It suggests that hyperlipidaemia is not a significant risk factor for cardiovascular disease in our patients with Fabry disease. On the contrary, high HDL-cholesterol may protect them from sustaining myocardial infarction or stroke. The measurement of apolipoprotein ApoA would be indicated to assess the load of these particles to confirm our hypothesis. In addition, the incidence of cardiovascular events among our cohort was rare apart from arrhythmia and stroke-like episodes in two cases. None of patients was treated with statins that reduced bias in the interpretation of lipid results. There was no predominant genotype among our male and female patients that would be associated with cardiovascular events.

Interestingly, some other lysosomal storage disorders are associated with low plasma HDL-cholesterol concentrations. Serum HDL-cholesterol concentration was found to be low in Gaucher disease patients and increases towards normal levels with ERT [24]. Niemann Pick A, B and C patients have been shown to have low HDL-cholesterol concentrations [26,27]. It was further supported by an experimental study that showed cells derived from sphingomyelinase knockout mice were defective in their ability to efflux cholesterol to apoA-I, emphasising that sphingolipid storage can contribute to the inhibition of cholesterol efflux [27,28].

One of limitations of the study was incomplete set of lipid profile at several time points. As a result, we did not include triglycerides or LDL-cholesterol in the analysis. Due to small numbers the P value was not included. In addition, non-fasting status often determines the choice of the limited lipid profile that contains only total cholesterol, HDL-cholesterol and total cholesterol/HDL-cholesterol.

In conclusion, patients with Fabry disease have raised HDL-cholesterol and, as a result, raised total cholesterol irrespective of the treatment option. The incidence of cardiovascular disease among these patients is known to be increased but it remains unclear whether it is related to the accumulation of glycosphingolipids or cholesterol in the

Table 2A
Female vs male patients on ERT: total cholesterol at baseline and at 6, 12, 24, 36, 48, 60 and 120 months of ERT. Normal cut off value for total cholesterol is < 5 mmol/L.

Mean total cholesterol (± SD)	Baseline	6 months	12 months	24 months	36 months	48 months	60 months	120 months
Females ERT (n = 23)	5.16 ± 0.7	4.9 ± 0.5	5.4 ± 1	5.1 ± 1.16	5.11 ± 1	5.12 ± 0.97	5.32 ± 1	5.33 ± 1.2
Males ERT (n = 32)	4.2 ± 0.7	4.5 ± 0.7	4.5 ± 0.8	4.43 ± 0.8	4.5 ± 0.9	4.7 ± 0.9	4.5 ± 0.8	4.5 ± 0.7

Table 2B
Female vs male patients on ERT; HDL-cholesterol at baseline and at 6, 12, 24, 36, 48, 60 and 120 months of ERT. Normal HDL-cholesterol cut off is > 1.1 mmol/L for men, > 1.2 mmol/L for females.

Mean HDL-cholesterol (± SD)	Baseline	6 months	12 months	24 months	36 months	48 months	60 months	120 months
Females ERT (n = 23)	1.76 ± 0.5	1.6 ± 0.1	1.8 ± 0.5	1.8 ± 0.46	1.84 ± 0.4	1.9 ± 0.5	1.9 ± 0.5	1.8 ± 0.5
Males ERT (n = 32)	1.5 ± 0.4	1.5 ± 0.3	1.6 ± 0.4	1.5 ± 0.4	1.5 ± 0.4	1.6 ± 0.6	1.56 ± 0.6	1.5 ± 0.5

arteries or both. Further studies, including carotid intima thickness measurement and apolipoprotein ApoA estimation, are required to examine the significance of raised HDL-cholesterol in patients with Fabry disease.

Conflict of interest

KS received travel grants from Genzyme, Alexion, Shire and Amicus. No conflict of interest for this publication.

CH is a Consultant for Actelion, Biomarin, Chiesi Inventiva, Sanofi, Genzyme and Shire and is owner director of FYMCA Medical Ltd. No conflict of interest for this publication.

Contributions

KS analysed all data and prepared the first draft of the manuscript.

CH initiated this project and contributed to the final version of the manuscript.

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