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ORIGINAL ARTICLE

Effect of lipid profile on prognosis in the patients with amyotrophic lateral sclerosis: Insights from the olesoxime clinical trial

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Abstract

Patients with ALS may have insufficientenergy substrates, due to dysphagia and hypermetabolism, which adversely affects the prognosis. Hyperlipidaemia has been reported to be associated with ALS and to represent a significant prognostic factor for survival in ALS. The aim of this study was to examine the prevalence of dyslipidaemia among a cohort of patients with ALS and how the lipid profile of patients with ALS influenced the prognosis. This was a prospective observational cohort study comprising 512 ALS patients, recruited for the TRO19622 (Olesoxime) investigational medicinal product trial. Fasting serum concentrations of total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) were measured as part of routine monitoring in the trial. Seventy-three percent of the participants had hypercholesterolaemia (defined as total cholesterol ≥ 5.1 mmol/l) at the screening visit. The prevalence of hypercholesterolaemia decreased with time and was 64% at 18 months follow-up. On unadjusted analysis total cholesterol, LDL-C and LDL/HDL ratio had a statistically significant effect on survival ($p = 0.015$, 0.003 and 0.027, respectively). When adjusted for prognostic covariates, however, none of the lipid measures was found to have a statistically significant effect on survival. In conclusion, prognosis in ALS is not influenced by the lipid profile of patients.

Key words: *Amyotrophic lateral sclerosis, lipid profile, survival, prognosis, metabolism*

Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative condition causing significant disability and shortened life expectancy (1). Progressive degeneration of both upper and lower motor neurons and denervation atrophy of the bulbar and skeletal muscles are the predominant features of the disease. In 50% of patients the life expectancy is 2–3 years from symptom onset, with death usually resulting from neuromuscular respiratory failure. The exact aetiology and pathophysiology of ALS is unknown but is believed to be a complex interplay of various environmental and genetic risk factors and multiple pathological processes (2). There is no cure and the only disease modifying therapy, riluzole, has a modest effect on the course of the disease. Supportive measures may help to improve the outcome. In this regard, nutritional management is an important factor. The body mass index (BMI) at diagnosis is an established prognostic factor in ALS and rapid weight loss is associated with a poor outcome (3,4). Patients with ALS are particularly predisposed to malnutrition for a variety of reasons that include dysphagia, fear of choking and aspiration, inability to feed themselves and high resting metabolic rate (5,6). Hence, various metabolic pathways are likely to be activated in such patients to provide energy substrates e.g. gluconeogenesis, lipolysis, and ketogenesis.

A high prevalence of hyperlipidaemia and its neuroprotective effect has been reported in ALS; however, this is not a consistent finding. The exact cause of hyperlipidaemia in ALS is uncertain: it may represent a compensatory mechanism in established disease; or a by-product of the underlying pathological processes, in particular mitochondrial dysfunction (7). The possibility of hyperlipidaemia

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being a compensatory mechanism is supported by the fact that lipids are a preferred source of energy for skeletal muscles and the regenerative effort of denervated muscles may cause an increased energy demand in ALS. Few studies have reported a possible neuroprotective benefit of hyperlipidaemia in ALS (see Discussion) and, also, the use of statins in people with ALS is reported to be associated with increased rate of functional decline (8).

It would be useful to study the prevalence of hyperlipidaemia in the ALS population, early in the disease course and with disease progression. A high prevalence of hyperlipidaemia, compared to the general population, at the time of diagnosis and with disease progression may indicate a role as a defence mechanism, if associated with improved survival. This study aimed to address this question, which has important clinical consequences in the nutritional management of patients with ALS. A secondary aim was to assess the effect of olesoxime, a cholesterol-like molecule, on cholesterol metabolism.

Methods

Study design

This was a prospective observational cohort study, using the clinical data base of the TRO19622 (Olesoxime) investigational medicinal product trial (9). This trial involving over 512 patients with ALS was conducted across 15 European centres (2009–2011). The patients were followed up at three-monthly intervals for 18 months while monitoring various biochemical and haematological parameters, including the lipid profile of each patient. It was a negative trial, with no survival benefit demonstrated in the treatment arm.

The primary research question asked whether hyperlipidaemia is associated with a better prognosis in patients with ALS.

All study sites obtained approvals from the local ethics committees. In the UK, the study protocol was approved by the National Research Ethics Service, Cambridge. Written informed consent was obtained from all patients (or carers of patients) participating in the study.

All study participants were diagnosed with ALS as per El Escorial criteria (definite ALS, probable ALS or probable laboratory-supported ALS). All participants had ALS related symptoms for more than six months and less than 36 months. Patients with a serious concurrent disease were excluded.

Blood biochemistry tests were performed at ICON Central Laboratories Dublin, Ireland. All laboratory results are reported in standard International Units or in conventional units. Blood samples were collected and shipped as per laboratory instructions. Patients were instructed to have fasted for six hours on the day of blood sampling. Serum was used for the lipid assays.

Statistical analysis

The statistical analysis was performed using software STATA, version 12 (10). The prevalence of hypercholesterolaemia was compared to the general population using the standardized incidence ratio (11). The relationship between weight and total cholesterol over time, adjusting for height at baseline, was modelled using a marginal model with Generalized Estimating Equations (GEE), exchangeable correlation matrix and robust standard errors. The relationship between longitudinal lipids data and overall survival was assessed using time-dependent Cox regression analyses. Each lipid parameter was regressed separately (due to the strong correlation between some of the measurements of lipid metabolism) against survival both unadjusted and adjusted for prognostic covariates.

Results

Basic demographics

The characteristics of the study participants are presented in Table I. The average age was 55 years, 65% were male and participants were predominantly sporadic MND with limb onset. The mean duration between disease onset and study entry was 27 months (range 10 months to 4.3 years).

Baseline lipid profile data

Table II gives the baseline lipid profile data of the study participants.

Prevalence of hypercholesterolaemia in the study cohort

Three hundred and seventy-six (73.5%) participants were classified as having hypercholesterolaemia at baseline, which is slightly (but not statistically significantly) higher than expected based on the normative population data (Health Survey England 2008) (expected age and gender matched prevalence 70%, prevalence ratio of hypercholesterolaemia (observed/expected) = 1.05 (95% CI 0.95–1.16) $p = 0.12$). As expected the prevalence declined over the study period due to disease progression, with prevalence being 64% at 18 months follow-up.

Relationship between BMI and total cholesterol during the period of follow-up

Figure 1 examines the relationship between BMI and total cholesterol over time. There appears to be no relationship between BMI and total cholesterol at any follow-up time-point.

Trend of weight and total cholesterol between the placebo and interventional group

Figure 2 illustrates the trend of weight and total cholesterol over time split by the placebo and

Table I. Characteristics of the study participants ($n = 512$).

Variable	*n*
Number of patients	512
Gender (M:F)	331 (65%):181 (35%)
Mean age (years) ± SD (range)	55 ± 11.3 (25 – 78)
Sporadic / Familial ALS	495 (97%):17 (3%)
Site of onset	
Bulbar	101 (19.7%)
Upper limb	200 (39.1)
Lower limb	206 (40.2%)
Cervical (presenting as head drop)	5 (1%)
El Escorial category	
Definite ALS	108 (21.1%)
Probable ALS	286 (55.9%)
Probable ALS - laboratory supported	118 (23%)
Mean weight (kg) ± SD (range)	71.9 ± 12.8 (38 – 127)
Mean BMI ± SD (range)	24.7 ± 3.6 (15.4 – 42.2)
Mean FVC (% predicted) ± SD (range)	93.3 ± 14.7 (69 – 156)
Mean ALSFRS-R score ± SD (range)	38.6 ± 5.03 (19 – 48)
Mean Manual Muscle Test score ± SD (range)	127.1 ± 18.4 (49 – 150)
Mean diagnostic delay (days) ± SD (range)	288 ± 193 (0 – 1078)
Deceased	
Total (% of cohort)	159 (31%)
ALS related (% of total deaths)	151 (95%)
Non-ALS related (% of total deaths)	8 (5%)
Mean disease duration (days) ± SD (range)	824 ± 286 (310 – 1557)

interventional groups. Weight declined steadily between one and 12 months in both groups. The overall trend of the total cholesterol levels is also downwards. The analysis demonstrated that total cholesterol decreases by 0.024 mmol/l for every 1-kg decrease in weight (95% CI 0.016–0.032, $p < 0.001$). Pre-treatment total cholesterol was insignificantly higher in the olesoxime arm (mean = 5.98 mmol/l) compared to the placebo (mean = 5.81 mmol/l) ($p = 0.07$), but the rate of decline was similar between the two groups, suggesting that olesoxime did not significantly affect cholesterol metabolism. Interestingly, pre-treatment weight was higher by 1 kg in the placebo group, once again demonstrating lack of relationship between weight and total cholesterol.

Effect of lipid profile on survival

We examined the effect of serial lipid profile over time on survival in this cohort of ALS patients (Table III). Total cholesterol, LDL-C and LDL/HDL ratio had a statistically significant effect ($p < 0.05$) on survival. However, this effect was not statistically significant when adjusted for prognostic covariates (age at disease onset, site of disease onset, forced vital capacity (% predicted), weight, gender and diagnostic delay). Gender was added as a covariate as it may affect cholesterol metabolism. Triglycerides seem to have the largest adjusted effect on survival, but none was statistically significant. Amongst the confounders, age at diagnosis, % forced vital capacity and weight had the most significant effect on survival ($p < 0.05$).

Figure 3 compares the mean cholesterol of the deceased patients with that of surviving patients. There is no significant difference between the two trajectories.

Discussion

Cholesterol is an essential constituent of cell membranes and is a precursor molecule in the synthesis of steroid hormones. LDL is the soluble form of cholesterol, which provides cholesterol to the tissues. Triglycerides function as energy reservoirs, stored in adipose tissue and provide energy in starvation/

Table II. Baseline lipid profiles ($n = 511$, unit mmol/l).

Lipids mmol/l (normal value)	Mean ± SD (range)	No. (%) outside normal range
Total cholesterol (< 5.18)	5.9 ± 1.0 (2.2 – 9.2)	376 (73%)
HDL cholesterol (≥ 1.04)	1.5 ± 0.4 (0.6 – 3.0)	51 (10%)
LDL cholesterol (< 2.59)	3.8 ± 0.9 (0.78 – 6.5)	455 (89%)
LDL/HDL ratio	2.7 ± 1.0 (0.7 – 6)	256 (50%)
Triglycerides (< 1.70)	1.6 ± 1.0 (0.4 – 16.3)	163 (32%)
BMI (25 Kg/m^2)	24.74 ± 3.64 (15.4 – 42.2)	212 (41%)

(Normal range in mmol/l: TC - < 5.18, HDL-C ≥ 1.04, LDL-C < 2.59, LDL/HDL ratio ≤ 2.4, triglycerides < 1.70).

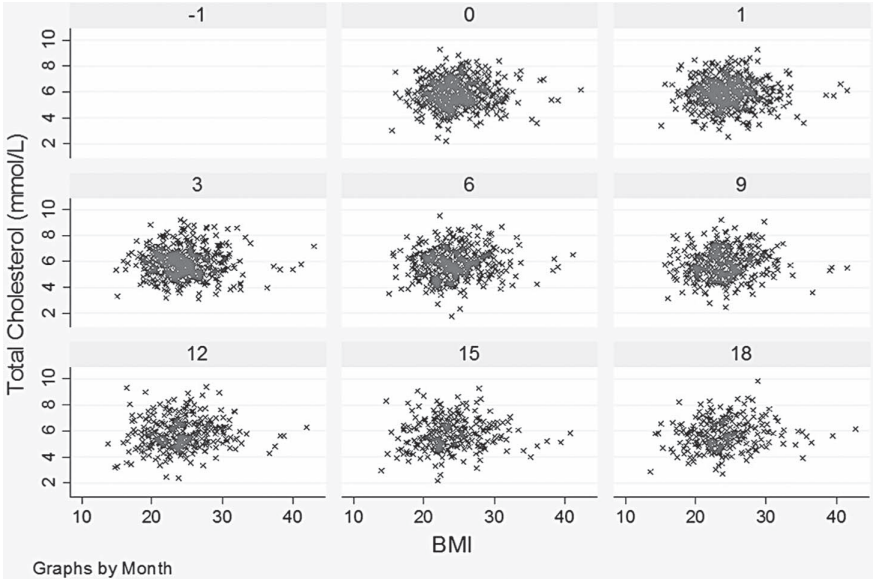

Figure 1. Scatter plots of total cholesterol against BMI at each follow-up time-point.

semi-starvation conditions. HDL transports endogenous cholesterol from the tissues to the liver, from where cholesterol may be excreted into the bile. HDL is associated with cardiovascular health.

A number of studies have evaluated the prognostic value of hyperlipidaemia in patients with ALS (Table IV). A potential survival benefit of hyperlipidaemia was first reported in 2008 in a French cohort of 369 patients with ALS and 286 healthy controls (12). The TC and LDL-C were two-fold higher in patients with ALS than in control subjects. Correlation studies demonstrated that an abnormally elevated LDL/HDL ratio improved survival by more than 12 months. The survival analysis was not adjusted for prognostic confounders. A German study reported a higher prevalence of hypertriglyceridaemia in ALS patients compared to the general German population. The survival benefit was, however, not significant on multivariate analysis (13). An Italian study refuted both of these observations and reported no difference in the prevalence of hyperlipidaemia in ALS patients and no effect on survival (14). Another study from the Netherlands reported a slightly improved survival in patients with high LDL/HDL ratio on univariate analysis; however, on adjusted analysis no difference was observed (15). Differences in genetic background and dietary habits in different populations may explain these discrepancies. Also, one should be aware of differences in defining dyslipidaemia (cut-off values) and laboratory enzymatic assays used in various publications, and the reported prevalence of dyslipidaemia may not be comparable in different studies. Future studies should pay particular attention to defining dyslipidaemia on an internationally acceptable standard (for example, using the WHO definition) and should present analysis having adjusted for prognostic confounders. Covariate adjustment is important in making comparisons of two different ALS cohorts as it is a heterogeneous condition with multiple prognostic factors.

Our study is unique for having longitudinal lipid profile data and in employing a time-dependent Cox proportional hazards model for survival analysis.

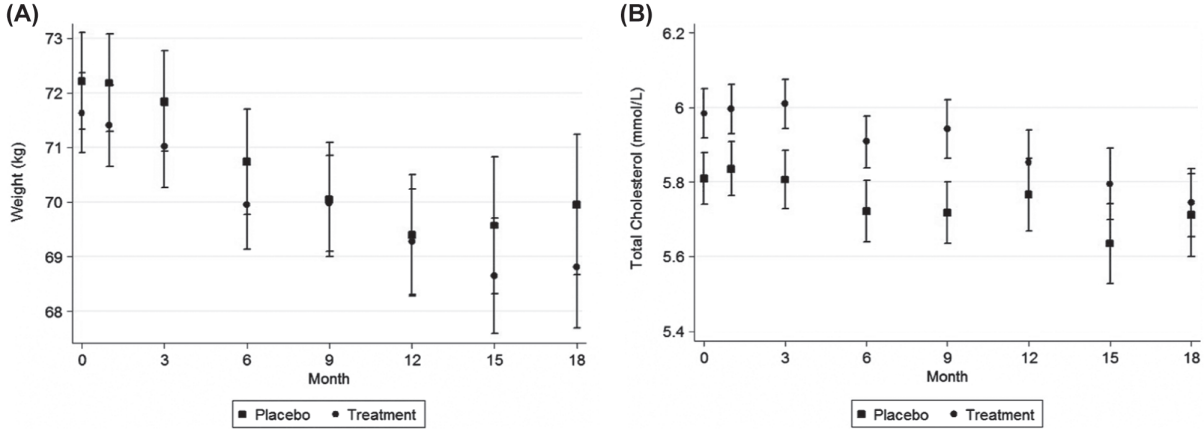

Figure 2. Plot of A) mean weight and B) mean total cholesterol (+/– SE) over time between the two groups.

Table III. Results from unadjusted and adjusted time-dependent Cox proportional hazards regression.

	Unadjusted ($n = 508$, $e = 157$)		Adjusted* ($n = 506$, $e = 141$)	
Lipid	Hazard ratio (95% CI)	p-value	Hazard ratio (95% CI)	p-value
HDL Cholesterol	1.07 (0.74 to 1.55)	0.731	0.70 (0.44 to 1.11)	0.131
LDL Cholesterol	0.79 (0.68 to 0.92)	0.003	0.92 (0.77 to 1.08)	0.305
LDL/HDL ratio	0.83 (0.71 to 0.98)	0.027	1.06 (0.88 to 1.27)	0.444
Total Cholesterol	0.84 (0.74 to 0.97)	0.015	0.93 (0.80 to 1.08)	0.336
Triglycerides	0.98 (0.85 to 1.14)	0.829	1.13 (0.97 to 1.31)	0.095

e: number of deaths; *adjusted for age, weight, gender, site of onset, disease duration and %FVC.

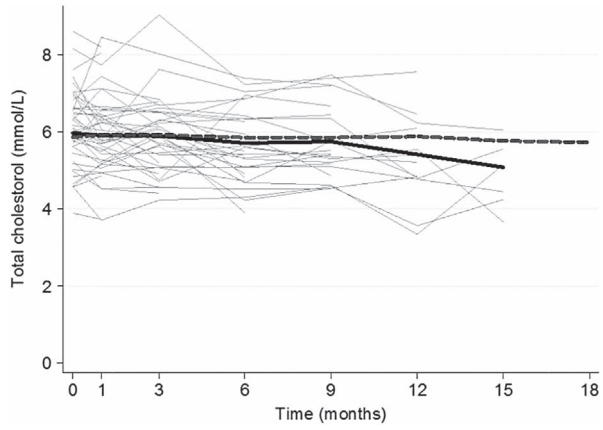

Figure 3. Spaghetti plot. Individual trajectory of total cholesterol for all those who died; their mean is the solid black line. The reference line (dashed) is the mean cholesterol of those who remained alive at the end of the trial period.

Our study did not show any significant link between serum lipid levels and survival in ALS, when adjusted for prognostic confounders. In agreement with the German study, our study demonstrated that out of all lipid measures triglyceride levels had the largest (but insignificant) effect on survival. This observation fits with the physiology of metabolism, as triglycerides function to provide a highly efficient form of energy in starvation or semi-starvation conditions. The importance of this observation is further reinforced by the fact that a higher BMI (which reflects the amount of stored triglycerides) is consistently reported to be a positive prognostic factor in ALS (16). Weight loss is a poor prognostic marker in ALS. A gradual decline in weight and total cholesterol with progression in the disease (Figure 2) highlights that attention should be given to careful nutritional support for patients with ALS. The declining trend of total cholesterol with disease progression is a novel finding and suggests that even if hyercholesterolaemia is assumed to be a protective metabolic parameter, it cannot be sustained with advancing disease in the absence of external nutritional intervention.

Table IV. Previous studies examining lipid profiles in ALS.

Reference	Total no. of patients with ALS	Outcome 1	Outcome 2
Dupuis et al. 2008[12] (French)	369	TC and LDL-C was two-fold higher in ALS patients then in matched controls	Raised LDL/HDL ratio increased survival by 12 months on univariate analysis.
Chio et al. 2009[14] (Italian)	658	Mean TC, LDL-C, HDL-C, triglycerides and LDL/HDL ratio was similar in patients with ALS and controls.	No association between lipid levels and survival on adjusted analysis. The mean TC and LDL-C was significantly lower in patients with FVC < 70% predicted.
Dorst et al. 2010[13] (German)	488	Hypertriglyceridaemia was more common in ALS patients compared to general German population	Prolonged life expectancy in ALS patients with higher triglyceride and cholesterol levels (not significant on multivariate analysis).
Sutedja et al. 2010[15] (Dutch)	303	Patients with ALS had a lower BMI and lower LDL/HDL ratio then controls.	A high LDL/HDL ratio correlated with increased survival which disappeared on adjusted analysis. The mean TC and LDL-C was significantly lower in patients with FVC < 70% predicted.
Paganoni et al. 2011[16] (USA)	427	BMI is an independent prognostic factor for survival, with the highest survival at 30–35 Kg/m^2.	LDL/HDL ratio was not associated with survival benefit.
Ikeda et al. 2012[21] (Japanese)	92	Significantly increased TC, LDL-C and triglycerides in female ALS patients.	Decline of ALSFRS and FVC was inversely correlated with TC and LDL-C.

Our cohort comprises a heterogeneous population from Belgium, France, Germany, Spain and the UK, i.e. a mixed population with different genetic and environmental backgrounds. The results describing the prevalence of hypercholesterolaemia need to be interpreted with caution as we did not have matching healthy control groups and the epidemiological data used for comparison were not strictly matched for geography and other variables. However, the World Health Organization MONICA (Multinational MONItoring of trends and determinants in CArdiovascular disease) project did not report any significant difference in the mean total cholesterol in the above mentioned European countries (range 5.6–6.1 mmol/l) (17).

There was no relationship between total cholesterol and BMI in the study cohort. This observation is supported by the MONICA project, which demonstrated only a weak correlation between TC and BMI (17). Another study using the same MONICA data set demonstrated that the prevalence of hypercholesterolaemia increases with advancing age (18). Hence, the high prevalence of hypercholesterolaemia in a relatively lean (mean BMI, 24.74) patient population of mean age 55 years, would not support the argument of hypercholesterolaemia being unique to ALS. Also, as there is no relationship demonstrated between the BMI and total cholesterol, the prognostic benefit of a raised BMI in an individual with ALS is likely to be independent of the lipid profile.

Although high blood lipid levels may not be associated with a better prognosis in ALS, the benefit of a high-fat diet cannot be excluded. Evidence from animal models of ALS suggests that a high-fat diet ameliorates the neurological deficits and improves survival (19). Moreover, mice fed on a high-fat diet lived longer than mice fed on diets containing high protein or high sugar. Studies are under way to evaluate the efficacy of a high-fat/high-calorie diet in people with ALS (20). Another phase I/II study is evaluating the safety and tolerability of a ketogenic diet in ALS (NCT01035710). It is proposed that ketones generated from ketogenic diet may ameliorate mitochondrial dysfunction and in so doing may preserve motor neuron function. Both of these studies offer late intervention, with a gastrostomy tube in place being a pre-requisite. Studies evaluating the therapeutic effect of early intervention with a high-fat diet in ALS are desirable.

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Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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