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LIPID PROFILE AND LIPOPROTEIN (A) LEVELS IN COQ10 SUPPLEMENTED ATORVASTATIN TREATED HYPERLIPIDAEMIC MYALGIA PATIENTS

Biochemistry

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ABSTRACT

Statins are the most efficient drugs for reducing plasma LDL-C level and generally well-tolerated and adverse effects can be reduced by co-administration of coenzyme Q_{10} (CoQ_{10}). In this study, 200 healthy normal subjects were taken as controls and 200 hyperlipidaemia subjects. From hyperlipidaemia subjects, 100 were treated with 40 mgs atorvastatin, 60 were atorvastatin treated hyperlipidaemic myalgia patients, out of 100 atorvastatin treated; 50 patients were atorvastatin treated hyperlipidaemia without CoQ10 and 50 patients were with CoQ10 supplementation. From atorvastatin treated hyperlipidaemic myalgia patients, 30 patients were without CoQ10 supplementation and 30 patients were with CoQ10 supplementation. Serum lipid profile and Lp(a) levels were measured before and after four weeks of treatment and protective effects of Co-Q_{10} was assessed. The results showed that atorvastatin significantly decrease lipid parameters and Lp(a) levels. Lipid parameters and Lp(a) levels in Co-Q_{10} supplemented groups were significantly lower than only statins administered groups. Our study provided new evidence supporting beneficial effects of Co-Q_{10} on statin-induced myopathic patients.

KEYWORDS

Hyperlipidaemia, Coenzyme Q10, Statins, Rhabdomyolysis.

INTRODUCTION

Hyperlipidaemia is one of the risk factor for cardiovascular disease and also induce disorder of lipoprotein metabolism, which includes a number of abnormalities such as hypercholesterolemia and hyper-triglyceridemia. Treatment of hyperlipidaemia with statins has become an integral part of management of vascular diseases (1, 2). HMG-CoA reductase inhibitors (statins) are efficient and safe means of reducing cholesterol and LDL levels (3, 4) and also due to up regulation of LDL receptor activity (5). Statin decrease cholesterol production by inhibiting the enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase), but the same biosynthetic pathway is shared by coenzyme Q10 or ubiquinone. Thus, both cholesterol and coenzyme Q10 biosynthesis decrease with statin treatment.

Coenzyme Q10 is an essential component of the mitochondrial electron transport system and deficiency of COQ10 may affect oxidative phosphorylation and mitochondrial adenosine triphosphate (ATP) production (6) which results from statin treatment therefore may impair muscle energy metabolism and contribute to the development of myopathy and muscle symptoms, described in patients treated with statins (7).

Lipoprotein (a) [Lp(a)] is a low-density lipoprotein (LDL)–like particle with apolipoprotein B covalently linked to apolipoprotein(a) by a single disulfide bond(8). Plasma Lp(a) is one of risk factor for cardiovascular disease. Whether increased Lp(a) is an additional risk factor for coronary artery disease in familial hypercholesterolaemia (9) is unknown. Lp(a) is similar to low density lipoprotein (LDL) in both lipid composition and the presence of apolipoprotein apoB-100. The difference between these two particles lies in the presence of the apo(a) moiety on Lp(a) (10). Although Lp(a) closely resembles LDL, its plasma concentrations and metabolism are quite distinctive.

Agarose gel electrophoresis has been widely employed by researchers to gain more knowledge of lipoprotein biology and its relationship to cardiovascular disease. Advances in this technique have been made in the visualization and quantitation of separated lipoproteins, detection and quantitation of apolipoproteins of the separated lipoproteins and in the detection of lipoprotein heterogeneity. Agarose gel electrophoresis helps to identify separation of LDL, VLDL, HDL and lipoprotein. Lp(a) shows an electrophoretic mobility clearly distinct from VLDL and HDL. Typically gel electrophoresis is a qualitative test, as the lipoproteins are stained with a dye such as Sudan black that primarily stains triglycerides and cholesteryl esters. The cholesterol content of the separated lipoproteins can, however, be quantitatively and enzymatically measured by a technique that treats the gel with cholesterol oxidase (11) (12).

2. MATERIALS AND METHODS

SAMPLE COLLECTION

Venous blood samples were drawn into sterile tubes (Vacutainer, BD, USA) with no additive and serum was separated by low-speed centrifugation. Measurement of general clinical chemistry parameters like Total cholesterol, HDL-C, LDL-C, VLDL-C (calculated), Triglycerides and Lp(a) were done using commercial kits from Siemens healthcare Diagnostics Inc (Tarry town, NY 10591- 5097, USA). Fully automated Siemens Advia 1800 clinical chemistry analyzer was used for this present study.

STUDY PROTOCOL

All participants in this study were of Indian origin. Apollo Hospitals Ethics committees approved the study protocols and all participants gave written informed consent. About 200 healthy normal subjects were taken as controls and 200 hyperlipidaemia subjects. From the hyperlipidaemia subjects, 100 were 40 mgs atorvastatin treated hyperlipidaemia patients, 60 were atorvastatin treated hyperlipi

daemic myalgia patients, Out of 100 atorvastatin treated hyperlipi daemic patients 50 patients were atorvastatin treated hyperlipidaemia without CoQ10 supplementation and 50 patients were atorvastatin treated hyperlipidaemia with CoQ10 supplementation. From atorvastatin treated hyperlipidaemic myalgia patients, 30 patients were without CoQ10 supplementation and 30 patients were with CoQ10 supplementation. These groups were considered for this study.

PROCEDURE

The hydrasys system is a semi automated multi-parameters instrument. The automated steps included processing of hydragel agarose gel in the following sequence: sample application, electrophoretic migration, dyeing, staining, destaining and final drying. The hydra gel was placed in applicator carrier on a flat surface and the part of applicator carrier with the numbered notches was raised. Distilled water 120 µl was pooled on the lower third of frame printed on the hydrogel K20 applicator carrier. Hydrogel agarose gel plate was unpacked. One thin filter paper was rolled quickly and uniformly onto the gel surface to absorb the excess of lipid. The paper was removed immediately. Gel plate was placed (gel side up) with edge against the stop at the bottom of the printed frame and the gel was bended and lowered down onto water pool. It was ensured that no air bubbles were trapped and water was spread underneath the entire gel plate and the gel was lined up with the printed frame. The applicator carrier was lowered with the numbered notches down to intermediate position with the switch in high position. One applicator was placed on a flat surface with the well numbers in right side of the position. Serum sample (10 µl) was applied into the applicator wells and the applicator was loaded within two minutes. The applicator teeth's protection frame was snapped off. Sample applicator was placed into position no. 4 on the applicator carrier. The applicator carrier was lowered with the switch so that the applicator contacts the gel surface.

After 7 min and 30 sec, the switch was turned to rise up the applicator, the applicator was removed and discarded. The gel was placed into an appropriate electrophoresis chamber, according to the polarity indicated on the gel, lower side of the gel on cathode side. When using SEBIA K20 chamber, the HYDRAGEL was placed on the bridge with the gel side facing down; the gel should dip about 1 cm into the buffer on the each side. After migration, the chamber was unplugged and the gel plate was removed. Gel was with hot 80 ˚C air (at least 45 minutes). The plate was placed into gel holder. The dried gel was immersed and cooled in the staining solution for exactly 15 minutes. De-stained for exactly 5 minutes in de-staining solution and the plate was placed into a gel holder. The gel shall immerse in the wash solution for exactly with distilled water. Excess liquid was drained vertically on the gel surface and the gel was dried with (80 °C hot air). If needed, back side of the dry film was cleaned with a tissue paper soaked with a 70% alcoholic solution. Using densitometer scanner the gel was scanned with at 570 nm.

STATISTICALANALYSIS

Statistical analysis data shown in tables are expressed as mean ± standard deviation. Differences between groups were examined for statistical significance using the T-test. P value less than 0.05 denoted the presence of statistically significant difference. All statistical tests were performed using the statistical package SPSS 16.0 version (SPSS Inc., Chicago, IIIinois, USA).

RESULTS

Table 1. Levels of lipid profile in control, hyperlipidaemia, atorvastatin and CoQ10 treated hyperlipidaemic myalgia patients.

Parameters	Control (n= 200)	Hyperlipidaemia patents (n = 200)	Atorvastatin treated hyperlipidaemia patients (n = 100)		Atorvastatin treated hyperlipidaemic myalgia patients (n = 60)		*p* Value
			Without CoQ10 (n = 50)	With CoQ10 (n = 50)	Without CoQ10 (n = 30)	With CoQ10 (n = 30)	
Total Cholesterol	165±29.7	285.0±41.8	175.87±62.2	171±59.4	169±30.21	164.2.±29.1	<0.001[abd] 0.004[c] 0.016[e]
HDL	46.3±9.13	41.2±11.8	45.81±10.4	47 .1±11.1	42.63±9.07	44.59±9.01	0.004[ab] 0.046[c] 0.011[d] 0.012[e]
LDL	93.4±22.6	167 ±43.8	129.16±44.5	124.2±41.2	95.25±29.8	91.21±25.15	<0.001[abd] 0.014[c] 0.017[e]
VLDL	25.2±11.7	66.8±58.8	34.90±24.0	33.2±20.1	21.88±16.3	21.1±16.1	<0.001[abd] 0.056[ce]
Triglycerides	104.70 ± 55.56	289.27 ± 109.28	191.30 ± 107.88	178.23±98.15	162.40 ±90.80	154.25±86.41	<0.001[abd] 0.003[ce]
Lp(a)	22.4±2.51	29.4 ±2.97	27.33±3.1	26.1±2.78	26.06±3.6	24.2±4.37	<0.001[abd] 0.007[c] 0.006[e]

HDL-C = high density lipoprotein cholesterol; LDL-C = low density lipoprotein cholesterol; VLDL = very low density lipoprotein cholesterol; TC = total cholesterol; Lp(a) = Lipoprotein (a). n = Number; Values are expressed as mean ± SD; Total cholesterol, HDL-C, LDL-C, VLDL-C, triglycerides and Lp(a) values are expressed as mg/dl.

P value - <0.001 shows high significance; p value – 0.05 shows significance; p value – >0.05 shows non significance.

[a]P value – Hyperlipidaemia group compared with control subjects.
[b]P value – Atorvastatin treated hyperlipidaemia without CoQ10 group compared with hyperlipidaemia group.

[c]P value – Atorvastatin treated hyperlipidaemia without CoQ10 group compared with atorvastatin treated hyperlipidaemia with CoQ10 group.

[d]P value – Atorvastatin treated hyperlipidaemic myalgia without CoQ10 group compared with hyperlipidaemia group.

[e]P value – Atorvastatin treated hyperlipidaemic myalgia without CoQ10 group compared with atorvastatin treated hyperlipidaemic myalgia with CoQ10 group

Table.1 depicts the levels of lipid profile parameters among patients and control subjects. The mean value of total cholesterol, LDL-C, VLDL-C, TGs and Lp(a) of hyperlipidaemia patients were significantly high (p<0.001) when compared to control subjects, except HDL-C which was significantly lower when compared to control subjects. Atorvastatin treated hyperlipidaemia patients without CoQ10 and atorvastatin treated hyperlipidaemic myalgia patients without CoQ10 showed a significant decrease in total cholesterol, LDL-C, VLDL-C and TGs except HDL-C which was higher when compared to hyperlipidaemia patients.

Similarly, atorvastatin treated hyperlipidaemia patients with CoQ10 and atorvastatin treated hyperlipidaemic myalgia patients with CoQ10 showed a significant decrease in total cholesterol, LDL-C, VLDL-C and TGs, when compared to atorvastatin hyperlipidaemia patients without CoQ10 and atorvastatin treated hyperlipidaemic myalgia patients without CoQ10. But with CoQ10 supplementation HDL-C was non-significantly high when compared to without CoQ10 in both groups.

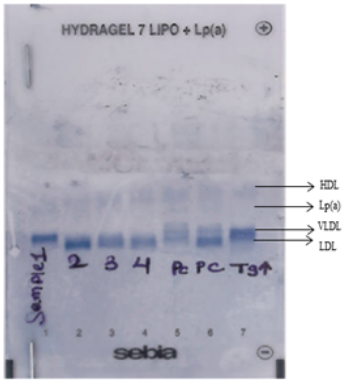

Fig. 1 Agarose gel (Hydrasys-Sebia) electrophoresis of Lp(a)

In this study, the mean value of Lp (a) was observed to be significantly ($p<0.001$) increased among hyperlipidaemia patients when compared to control subjects. But, atorvastatin treated hyperlipidaemia patients without CoQ10 and atorvastatin treated hyperlipidaemic myalgia patients without CoQ10 showed a significant ($p<0.001$) decrease in Lp(a) level when compared to hyperlipidaemia patients. Similarly, atorvastatin treated hyperlipidaemia patients with CoQ10 and atorvastatin treated hyperlipidaemic myalgia patients with CoQ10 showed a significant decrease in Lp(a) level when compared to atorvastatin treated hyperlipidaemia patients without CoQ10 and atorvastatin treated hyperlipidaemic myalgia patients without CoQ10.

DISCUSSION

Lp(a) has been identified as one of the independent risk factor for CVD. Several clinical trials, particularly with respect to the catabolism, pharmaceutical science has not yet developed drugs that are able to reduce elevated Lp(a) concentrations to the desired levels (13). This comparative study of statins was primarily aimed at studying the effect of statins on Lp(a) levels in Indian population. Performed a large cohort study, which showed a significant decrease in the Lp(a) concentration by atorvastatin treatment (14).

Figure-1 shows the lipoproteins separated to the following fractions like chylomicrons which are large molecules with high triglyceride content and present as a small particle in the serum and it normally remain at the sample application point. The beta lipoproteins or low density lipoproteins (LDL-C) normally migrate in beta 2 globulin positions and pre beta lipoproteins or very low density lipoproteins (VLDL-C) have high molecular weight and migrate in beta 1 globulins position. The fast pre beta lipoprotein is composed of the Lp(a) which is similar in size and composition to LDL-C. Lp(a) when present at sufficiently higher concentration, can been seen migrating between VLDL and HDL. The alpha lipoprotein or high density lipoprotein (HDL-C) are fastest fraction of lipoproteins. They are migrating to alpha 2 globulins positions (15) (16).

In this study, it is noteworthy that treatment with CoQ10 provided a significantly enhanced potency of statin with decreased serum lipid profile levels. The increases of TC, TG, LDL-cholesterol, VLDL-cholesterol and Lp(a) in hyperlipidaemia patients were significantly reduced by statin treatment. Moreover, the combined treatment of statin with CoQ10 further decreased TC, TG, LDL-C, VLDL-C and Lp(a) levels and significantly increased HDL-C levels (Table.1). These results are consistent with previous report that CoQ10 supplementation inhibits the peroxidation of LDL-C which may play a key role in its anti-atherogenic effects.

CONCLUSION

Serum Lp(a) is conveniently used for the diagnosis of primary hyperlipidemia and coronary heart disease. In this study, the significant reduction of total serum cholesterol, LDL-C, VLDL-C, Triglyceries and Lp(a) levels achieved represents the good result of the CoQ10 supplementation with atorvastatin therapy, were as a non-significant increase in HDL level was seen. The anti-atherogenic effects of CoQ10 supplementation are beneficial in statin treatment. Moreover, the possibility of a synergistic effect of statins with CoQ10 supplementation should be further evaluated for clinical purposes.

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