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FTIR ANALYSIS OF PLASMA COQ 10 IN ATORVOSTATIN TREATED HYPERLIPIDAEMIA PATIENTS WITH MYOPATHY

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ABSTRACT

Hypercholesterolemia is one of the leading causes of death and disability in the worldwide. Statins (HMG-CoA reductase inhibitors) are medications at the forefront of the battle against cardiovascular disease. Despite their effectiveness, patient compliance with statins has lagged because of adverse effects, namely myalgia, myopathy and rhabdomyolysis. Myopathy is the most common side effect of statin use. The purpose of this study is to report plasma levels of CoQ10 in patients taking statins and then to determine the benefit of Coenzyme Q10 (CoQ10) supplementation on statin-related myopathy as evidenced by symptomatic improvement and increase in serum levels of CoQ10.

Key words

Hypercholesterolemia, statins, myalgia, myopathy, rhabdomyolysis, CoQ10

Introduction

Cardiovascular disease (CVD) is an abnormal functioning of the heart or blood vessels. Impaired endothelial function followed by inflammation of the vessel wall leads to atherosclerotic lesion formation that causes myocardial infarction, stroke, hypertensive heart disease, heart failure, coronary artery disease and peripheral artery disease. The major cause for CVD risk factor includes Diabetes, hypertension, high cholesterol, obesity, lack of exercise, smoking, increased age and family history (Tortora *et al.*, 2014; Gaziano *et al.*, 2016). Cardiovascular diseases (CVDs) are the leading cause of death globally. An estimated 17.9 million people died from CVDs in 2019, representing 32% of all global deaths. Of these deaths, 85% were due to heart attack and stroke. Over three quarters of CVD deaths take place in low- and middle-income countries. Out of the 17 million premature deaths (under the age of 70) due to noncommunicable diseases in 2019, 38% were caused by CVDs. Most cardiovascular diseases can be prevented by addressing behavioural risk factors such as tobacco use, unhealthy diet and obesity, physical inactivity and harmful use of alcohol. It is important to detect cardiovascular disease as early as possible so that management with counselling and medicines can begin (WHO, 2019).

Cholesterol homeostasis is controlled mainly by endogenous synthesis, intestinal absorption, and hepatic excretion. An imbalance of these processes may lead to high cholesterol concentrations in the plasma, cholesterol accumulation in different tissues, and increased risk of atherosclerotic cardiovascular diseases (Park & Carr, 2013). Statins or 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors were the most effective drugs for cardiovascular risk reduction (Harper et al., 2010) and associated with primary and secondary prevention of coronary artery disease (McPherson et al., 2006; Sanjay et al.,2009). Statins have consistently been shown to reduce cardiovascular related mortality and morbidity through the reduction of low density lipoproteins cholesterol (Ward et al., 2007). Statins have become the second largest prescribed drugs class in the United States (Robin et al., 2013). In view of this, statins were considered a promising candidate for the treatment of CHD, because statins exert diverse cellular, cholesterol lowering effects

throughout the cardiovascular system improvement of nitric oxide synthesis, endothelial function and inhibition of inflammatory cytokines (Mancini *et al*., 2011; Mozaffarian *et al*., 2015).

Coenzyme Q1 (2, 3-dimethoxy-5-methyl-6-decaprenyl-1,4-benzoquinone), also known as ubiquinone, is a naturally occurring and extremely hydrophobic molecule, with characteristics common to vitamins. This substance is called CoQ10 as this compound has 10 isoprenoid units in the side chain (Wang *et al*., 2004). CoQ10 is widely found in living organisms, including yeast, plants, animals and humans (Chew *et al*., 2004). CoQ10 plays a critical role as an antioxidant and electron transfer in the mitochondrial inner membrane involved in the efficient production of high-energy phosphates necessary for muscle contraction and other cellular function (Littarru *et al*., 2007). Generally, every cell has the ability to synthesize CoQ10, but it is mostly synthesized in the liver.

A deficiency of CoQ10 can cause issues with heart health, metabolism and energy production (Dutton *et al*., 2000). CoQ10 is a key component of the mitochondrial respiratory chain. It participates in electron transport during oxidative phosphorylation in the mitochondrial muscle cells. Accordingly, one of the proposed theories for the cause of myopathy in statin drug users is that CoQ10 deficiency resulting from statin treatment may impair muscle energy metabolism and contribute to the development of myopathy in patients treated with statins (Bhagavan *et al*., 2006). Oral supplementation of CoQ1O has been shown to reverse the decrease in serum CoQ10 levels by statins.

A Fourier transform infrared (FT-IR) spectrometric method was developed for the rapid, direct measurement of plasma coenzyme Q_{10} (CoQ_{10}) in different groups.

Materials and Mathod

Study protocol

The study was carried out in accordance with Indian origin and approved by the Ethics Committee of the Apollo hospitals. Subject's participence were included in the study after their obtained informed consent, prior to explaining the nature and the purpose of the study. About 200 healthy normal subjects were taken as

controls, 200 hyperlipidaemia patients, 100 who were 40mg atorvastatin(zydus) treated hyperlipidaemic patients for three month, 60 were atorvastatin treated hyperlipidaemic myalgia patients. Out of 100 atorvastatin treated hyperlipidaemic patients, 50 patients who were atorvastatin treated hyperlipidaemia without CoQ10 supplementation, 50 patients were atorvastatin treated hyperlipidaemia with 300mg CoQ10(sanofi) supplementation for three month. From atorvastatin treated hyperlipidaemic myalgia patients, 30 patients were treated without CoQ10 supplementation, 30 patients were treated with CoQ10 supplementation and are considered for this study groups.

CoQ10 analysis using - FTIR (Fourier Transform Infrared Spectroscopy) method

CoQ10 was measured by FTIR method of Ciurczak E W, (2001).

Procedure

2 ml of blood samples were collected in EDTA vacutainer from patients. The blood was immediately centrifuged for 3 min to separate plasma from cells. The plasma samples were then stored at -20˚C before analyses. After the samples returned to room temperature (about 15 min at 25-30˚C), 1ml serum was diluted with an equal volume of aqueous potassium thiocyanate solution (4 mg/L). 20 µL of each diluted sample was spread evenly over the surface of a thallium chromide pellet. All the specimens were air dried for 30 minutes prior to measuring the spectra. The strong absorption band of water in the mid IR- region poses hindrance and hence to eliminate this, the serum samples were air dried. The dried serum forms a thin uniform film on the pellet. Infrared transparent thallium chromide without the sample was scanned as background for each spectrum and 16 scans were co-added at a spectra resolution of ± 1 $cm^{-1.}$

FT-IR spectra were recorded with different resolutions. The spectra were scanned between 4,000 and 400 cm^{-1}, by averaging 64 scans for each sample, with a resolution of 4 cm^{-1} (data point resolution/interval 1 cm^{-1}) and with a resolution of 8cm^{-1} (data point resolution/interval 2cm^{-1}) respectively. Accordingly, two

sets of spectra were obtained for each sample. Background spectra were obtained for each experimental condition.

Data analysis

All the data were expressed as mean and standard deviation are computered and compared between hyperlipidaemia patients and control group by student T-Test in Graph Pad Prism

Results

Level of CoQ10 status in control, hyperlipidaemia, Atorvastatin and CoQ10 treated hyperlipidaemic myalgia patients.

Parameters	Control (n= 200)	Hyperlipidaemia patients (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)	
CoQ10(mg/l)	0.75 ±0.06	0.74 ±0.08	0.59 ±0.08	0.71 ±0.10	0.48 ±0.07	0.69 ±0.09	NS[a] <0.01[bc] <0.001[de]

n = Number; Values are expressed as mean ± SD; CoQ10 values are expressed as mg/l

p value - <0.001 shows high significance; p value – 0.05 shows significance; p value – >0.05 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 grou

CoQ10 analysis using Fourier transforms infrared spectroscopy (FTIR) method. Coenzyme Q10 is a naturally occurring and fat-soluble quinone that is localized in hydrophobic portions of cellular membranes. Approximately half of the body's CoQ10 is obtained through dietary fat ingestion, whereas the remainder

results from endogenous synthesis (Crane et al., 1989). Coenzyme Q10 participates in electron transport during oxidative phosphorylation in mitochondria protects against oxidative stress produced by free radicals and regenerates active forms of the antioxidants ascorbic acid and tocopherol (vitamin E). Statins block production of farnesyl pyrophosphate, an intermediate in the production of CoQ10. This fact plus the role of CoQ10 in mitochondrial energy production and the importance of mitochondria in muscle function has prompted the hypothesis that statin-induced CoQ10 deficiency participates in statin-associated myopathy (James et al., 2004). Infrared (IR) spectroscopic method for the simultaneous determination of Total-cholesterol, TG, HDL-C and LDL-C. The pattern of IR absorption is exquisitely sensitive to both molecular structure and conformation. In analogy Quantification of LDL-C was derived from two spectral regions encomposing strong lipid absorptions, namely 1700-1800 cm-1, which contains the C=O stretching vibration and 2800-3000 cm-1 which includes the acyl CH2 stretching modes, The HDL-C quantification method required additional spectral information, namely the 900-1500 cm^{-1} for optimal performance. The spectral regions were identified as 1700-1800, 2800- 3000 cm^{-1} for total cholesterol and 2800-3200 for triglycerides by Shaw et al. In analogy with the above views, the absorption peaks at 3060, 2929, 1736 and 1446 cm-1 are assigned to TG, TC, LDL and HDL respectively. In this study, the area normalized amide I' bands 1800-1700cm^{-1} of LDL superimpose completely on the corresponding amide I' band of large buoyant LDL in control- 1746, hyperlipidaemia- 1655, atorvastatin treated hyperlipidaemia patients withoutCoq10-1741,1711, atorvastatin treated hyperlipidaemia patients with Coq10- 1742, atorvastatin treated hyperlipideamic myalgia patients without CoQ10- 1654, atorvastatin treated hyperlipideamic myalgia patients with CoQ10-1652.

Fig. 1 FTIR spectrum of control sample

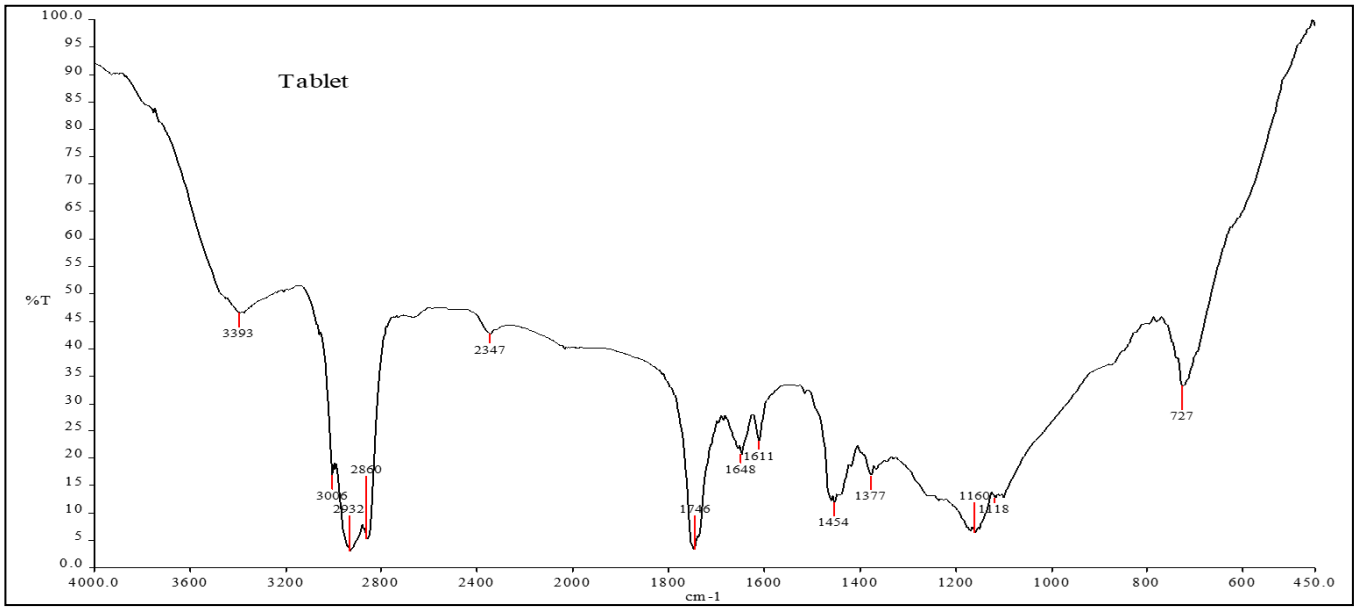


Fig. 2 FTIR spectrum of pre-treatment group

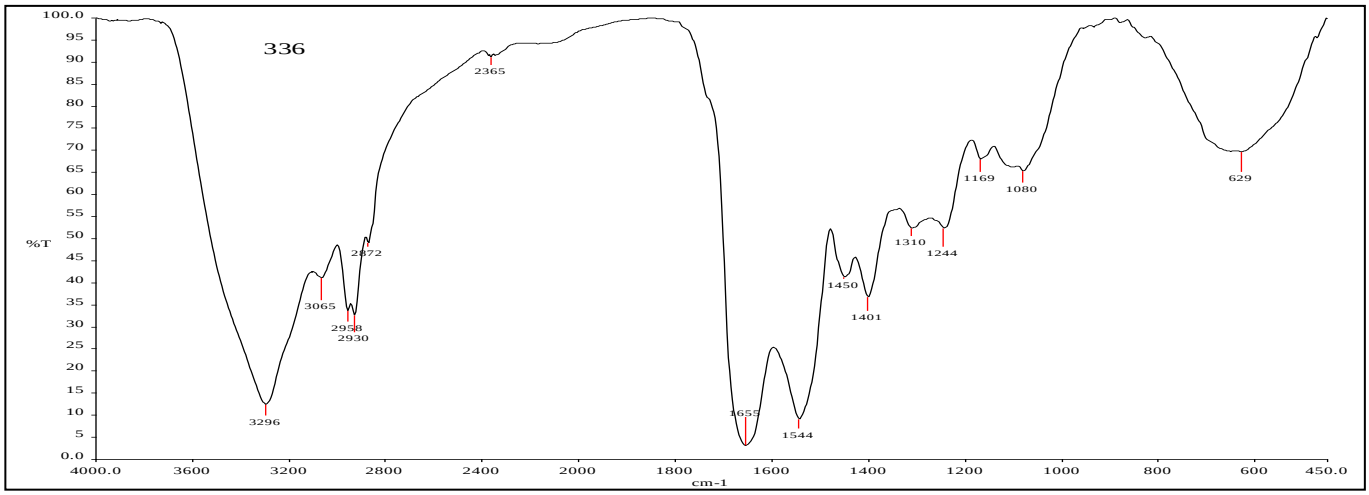


Fig. 3 FTIR spectrum of Atorvastain treated Hyperlipidaemia patients without COQ10

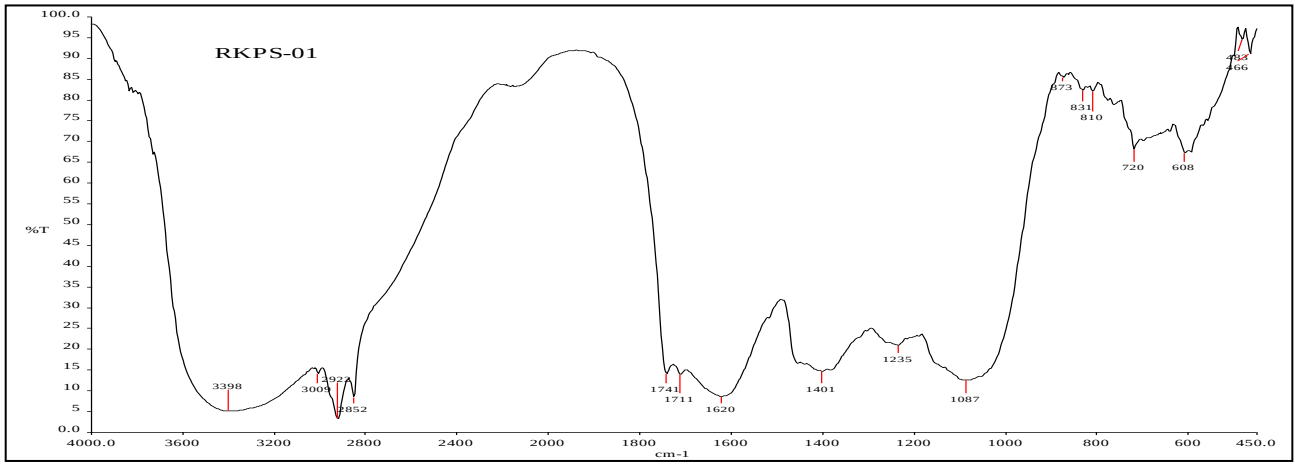


Fig. 4 FTIR spectrum of Atorvastain treated Hyperlipidaemia patients with CoQ10

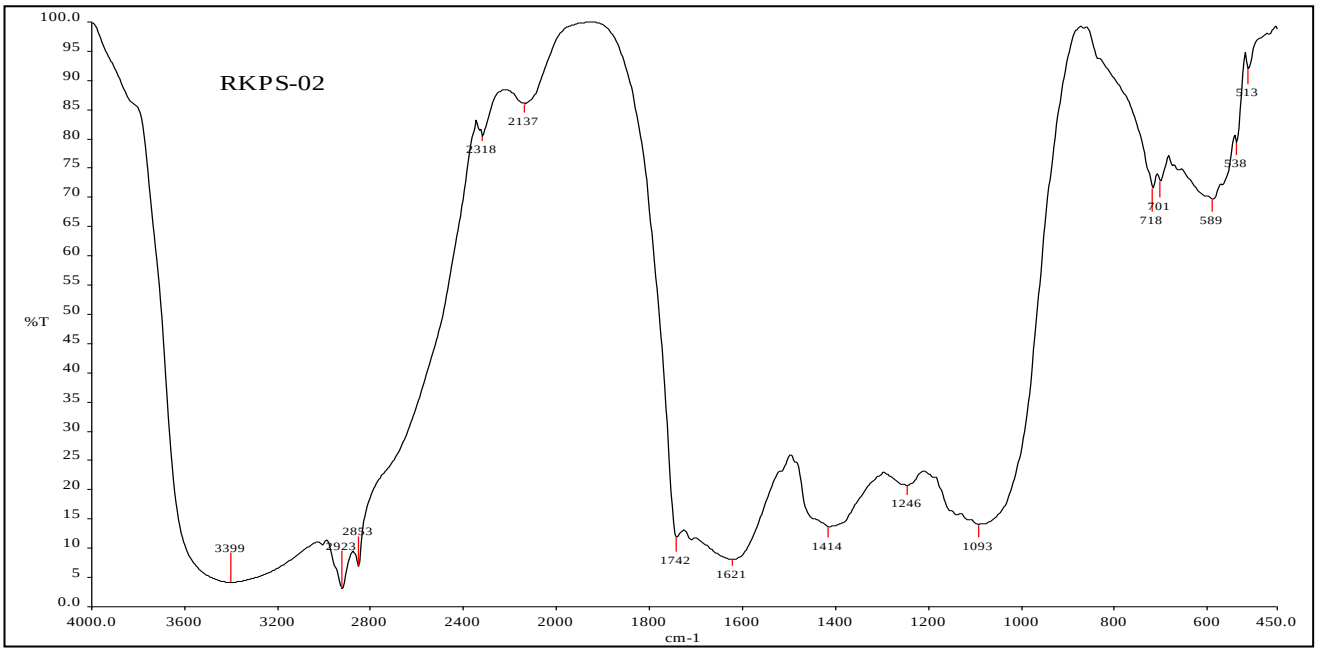


Fig. 5 FTIR spectrum of Atorvastain treated hyperlipidaemic myalgia patients without CoQ10

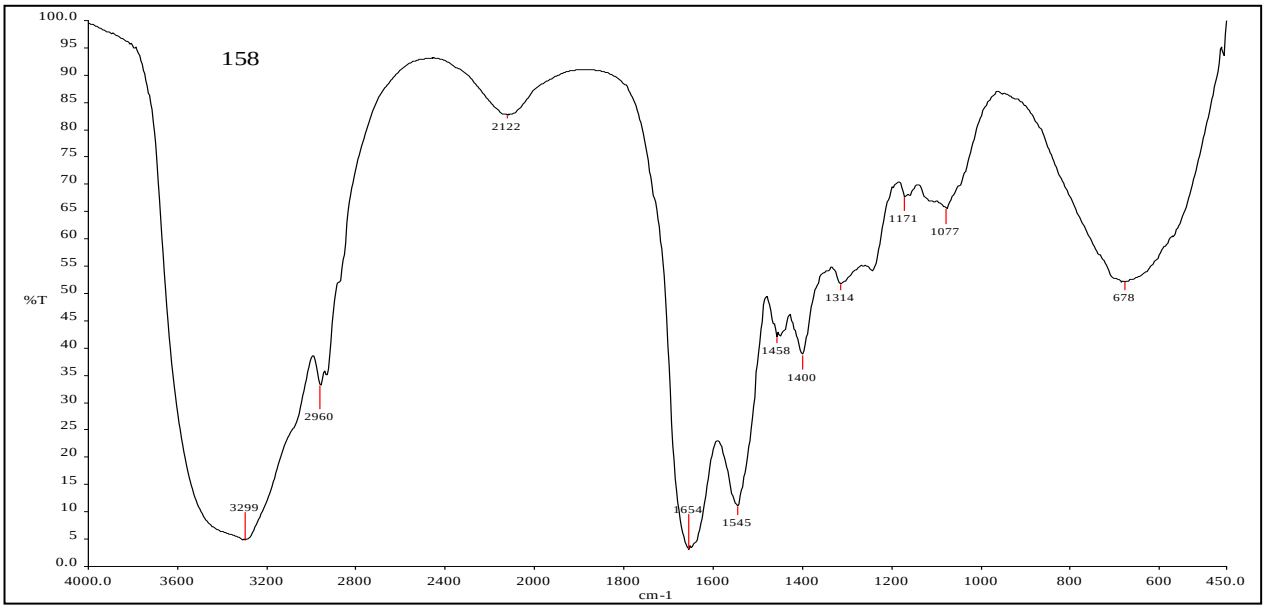
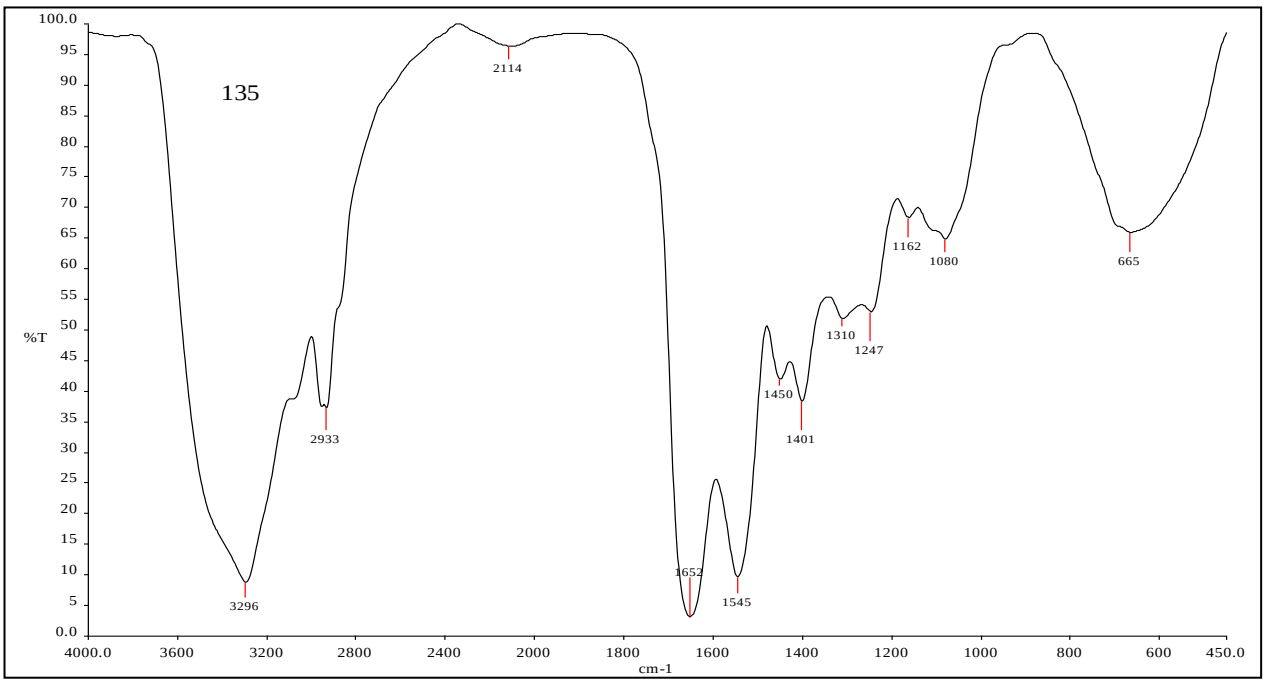


Fig.6 FTIR spectrum of Atorvastain treated hyperlipidaemic myalgia patients with CoQ10



Discussion

Statin medication is vital for the treatment of hypercholesterolmia as well as for primary and secondary prevention of cardiovascular disease and has been shown to reduce the risk of morbidity and mortality in coronary events. Statins are effective lipid lowering drugs that are commonly prescribed medication. These drugs are one of the most effective ways to reduce low density lipoprotein cholesterol levels and also reduce the other disorder such as cancer, stroke and inflammatory conditions. Therefore, the extended use of statin medication demands awareness, recognition and proper evaluation and treatment of myalgia and myopathy by healthcare providers.

Statins block production of farnesyl pyrophosphate, an intermediate in the production of CoQ10. Coenzyme Q10 is important compound of oxidative phosphorylation and it participates in electron transport during oxidative phosphorylation in mitochondria, protects against oxidative stress produced by free radicals (James *et al*., 2004). The primary adverse effect limiting their use is myopathy, ranging from benign myalgia to rare cases of fatal rhabdomyolysis (Staffa *et al*., 2002). In the present study is to use FT-IR spectrophotometry to quantify CoQ10 in plasma. In different kinds of muscle disease the beneficial effects of coenzyme Q10

supplementation have been shown to correlate with improvement in oxidative phosphorylation as monitored by FT-IR techniques and is used to analyze the control, hyperlipidaemia, atorvastatin treated hyperlipidaemia, atorvastatin treated hyperlipidaemic myalgia patients and with CoQ10 of atorvastatin treated hyperlipidaemia and atorvastatin treated hyperlipidaemic myalgia patients. In this study, atorvastatin treated hyperlipidaemia and atorvastatin treated hyperlipideamic myalgia patients showed a significance decrease in CoQ10 level.

Conclusion

The present study revealed that by coenzyme Q10 supplementation of the regular statin treatment, associated with muscle pain in the mild to moderate symptoms group could be significantly reduced. Consequently, it may lead to lower interference with daily activities and higher compliance with statin treatment. These observations mean a better quality of life, besides adequate cardiovascular protection, which is the primary goal of statin therapy. The results of the present study are very promising, but require further testing in larger clinical trials, mainly to allow them to be generalized.

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