University of Kerbala
College of pharmacy
Department of Pharmacology and Toxicology

Effect of COQ2 Gene Polymorphism on incidence of myopathy in patients treated with atorvastatin in Kerbala Province

A Thesis

Submitted to the Council of College of Pharmacy/University of Kerbala as a Partial Fulfillment of the Requirements for the Master Degree of Science in Pharmacology and Toxicology

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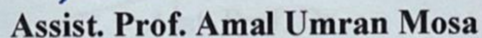

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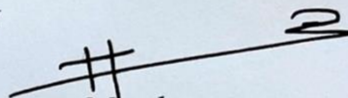

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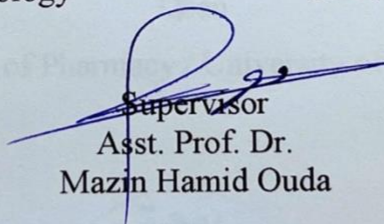

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DEDICATION

To...The Soul of My Father, Allah Almighty bless him....

To... My source of inspiration who helped me in every stage of my life... My kind mother...

To... My life partner "husband", whose constant encouragement, limitless giving and great sacrifice, helped me accomplish my degree and my dream.

To... My sisters and brother who shared their advice and encouragement with me to go on.

To... My dear Son, who have made me laugh, wiped my tears, hugged me tight and kept me strong.

Respect and Love...

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List of abbreviation

Abbreviation	Meaning
4- AAP	4-Amino-Anti Pyrine
ADP	Adenosine Di Phosphate
ARMS	Amplification Refractory Mutation System
ABC	ATP-Binding Cassette
ACC/AHA/NHLBI	American College of Cardiology/American Heart Association/National Heart, Lung, and Blood Institute
Apo B	Apolipoprotein B
BMI	Body Mass Index
CHOD	Cholesterol Oxidase
CE	Cholesterol Esterase
CoQ10	Coenzyme Q10
CABC	Chaperone-Activity of BC1
COQ2	Coenzyme Q2
CK	Creatine Kinase
CYP2D6	Cytochrome P450 Family 2 Subfamily D Member6
CYP3A4	Cytochrome P450 Family 3 Subfamily A Member4
CYP3A5	Cytochrome P450 Family 3 Subfamily A Member5
CPY2C9	Cytochrome P450 Family 2 Subfamily C Member9
CVD	Cardiovascular Diseases
DME	Drug Metabolizing Enzyme
DNA	Deoxyribonucleic Acid
decap- renyl-PP	Decaprenyl Diphosphate

ELISA	Enzyme-Linked Immunosorbent Assay
EDTA	Ethylenediaminetetraacetic Acid
F-PP	Farnesyl Pyrophosphate
GFR	Glomerular Filtration Rate
G6P-DH	Glucose-6-Phosphate Dehydrogenase
GTP	Guanosine Triphosphate
GG-PP	Geranylgeranyl Pyrophosphate
HMG CoA	3-Hydroxy-3-Methylglutaryl- Coenzyme A
HDL-C	High Density Lipoprotein Cholesterol
HK	Hexokinase
IF	Inner Forward
IR	Inner Reverse
IDL	Intermediate Density Lipoproteins
LDL	Low-Density Lipoprotein
MHC	Major Histocompatibility Complex
MDR1	Multidrug Resistence
NLA	National Lipid Association
OF	Outer Forward
OR	Outer Reverse
OATP1B1	Organic Anion Transporting Polypeptide 1B1
PEG	Polyethylene Glycol
POD	Peroxidase
PCR	Polymerase Chain Reaction
PDSS	Prenyldiphosphate Synthase Subunit
PP	Pyrophosphate
PHB	Para-Hydroxy-Benzoate

RR3	Ryanodine Receptors 3
SAMS	Statin Associated Muscle Symptoms
SNP	Single Nucleotide Polymorphism
SLCO1B1	Solute Carrier Organic Anion Transporter Family Member 1B1
TSH	Thyroid Stimulating Hormone
TBE	Tris/Borate/EDTA
ULN	Upper Limited of Normal
US FDA	United States Food and Drug Administration
VLDL-C	Very Low Density Lipoprotein Cholesterol
W1 buffer	Washing Buffer

Abstract

Background: The COQ2 gene 4-hydroxybenzoate encoding polyprenyltransferase (coenzyme Q2), belongs to the candidates potentially influencing statin treatment tolerability. This enzyme is involved in the biosynthesis of coenzyme Q10 (CoQ10), in which depletion induced by statin treatment is implicated in the development of statin-associated muscle symptoms (SAMS). Thus, polymorphisms in the COQ2 gene might explain susceptibility to statin induce myopathy. Myopathy is a major side effect of statins that leads to statin intolerance and discontinuation.

Aims of study: The aim of this study is to detect the role of genetic polymorphism of COQ2 gene particularly COQ2 (A>G) (rs6535454) and COQ2 (C>A) (rs6818847) that involved in the incidence of myopathy in Iraqi patients treated with 40mg atorvastatin.

Patients and methods: This cross-sectional observational study was done at Imam Al-Hussein Medical City in Kerbala. One hundred fifty patients with atorvastatin drug were selected to participate in this study. All patients enrolled in this study with age ranged (30-65) were treated with atorvastatin tablet (40mg) once daily. Blood samples were obtained from patients who had signed informed consent for genetic testing and it was used for measurement of Coenzyme Q10. lipid profile (cholesterol, triglyceride, low-density lipoprotein, and high-density lipoprotein), renal function test (creatinine), creatine kinase, and thyroid stimulating hormone. This study used Amplification Refractory Mutation System Polymerase Chain Reaction (ARMS PCR) for detection of COQ2 (A>G) (rs6535454) and COQ2 (C>A) (rs6818847).

Results: The obtained results from this study have detected multiple genotypes of COQ2 gene particularly COQ2 (A>G) (rs6535454) and COQ2 (C>A) (rs6818847), that include the homozygous wild genotype (AA), homozygous mutant (GG) and heterozygous (AG) genotype of COQ2 (A>G) (rs6535454) while for COQ2 (C>A) (rs6818847) that include the homozygous wild genotype (CC), homozygous mutant (AA) and heterozygous (CA) genotype detected in statin taking patients participated in this study. Regarding to the level of coenzyme Q10 and creatine kinase in the serum, the present study showed that significant association ($p>0.05$) between the studied SNPS of COQ2 gene and serum CoQ10 level, while non significant association ($p>0.05$) between the studied SNPs of COQ2 gene and serum creatine kinase level in the statin taking patients included in the study. Conclusion: In the Iraqi dyslipidemic patients treated with high dose of atorvastatin, there is a significant effect of common polymorphisms (rs6535454 and rs6818847) within the COQ2 gene, this poses a risk of developing myopathy associated with the use of atorvastati

CHAPTER ONE
INTRODUCTION

1. Introduction

1. 1 Myopathy

Myopathies are a diverse groups of heterogeneous disorders characterized mostly by abnormalities in skeletal muscle structure and function (1). They can develop as a result of endocrine, immunological, or metabolic diseases and can be genetic or acquired. Although they don't have any specific symptoms, myopathies often worsen over time, and symptoms like muscle weakness, fatigue, weakness, and movement restriction (which can affect different muscle groups depending on the myopathy form) can raise suspicions. The proximal muscles—those in the shoulder, pelvis, and upper thigh—are often more severely affected than the distal muscles in the early stages of the illness, resulting in symptoms like postural instability and the inability to lift the hands or stand up from a seated position. The severe hereditary myopathy known as muscular dystrophy is regarded to be more deadly than other myopathies, which eventually lead to muscle atrophy and force some people into wheelchairs (2).

1.2. Types of Myopathy

1.2.1. Inherited

Inherited myopathies include:

1.2.1.1. Mitochondrial myopathy

Caused by a deficit in the mitochondria, the cell's energy-producing organelle. Different forms of mitochondrial myopathy exist. While inherited mutations (abnormalities in the DNA) can cause them, they can also happen without any family history (3).

1.2.1.2. Metabolic myopathy

Caused by metabolic issues that affect how well the muscles work. Several hereditary metabolic myopathies are brought on by errors in the genes that produce specific enzymes necessary for healthy muscle function (4).

1.2.1.3. Nemaline myopathy

Characterized by the presence of "nemaline rods," or muscular structures, in the muscles. Weakness in the respiratory muscles is frequently correlated with nemaline myopathy (5).

1.2.2. Congenital

Congenital myopathies include:

1.2.2.1. Central core myopathy

A genetic myopathy, also known as central core disease, which results in brittle bones, weakness, and severe drug reactions. Some patients with this disease experience extreme weakness, while others only experience minor weakness (6).

1.2.2.2. Muscular dystrophy

These are brought on by muscle deterioration or abnormally generated muscle cells (7). Muscle loss and weakness occur gradually in a group of diseases known as muscular dystrophies. Muscular dystrophy is brought on by unusual genes (mutations) that interfere with the production of the proteins needed to build healthy muscle.

1.2.3. Acquired

Commonly acquired myopathies include:

1.2.3.1. Inflammatory/ autoimmune myopathy

When the body assaults itself, it can lead to muscle degeneration or function issues. This is the form of myopathy that results. Several myopathies, such

as polymyositis, dermatomyositis, sarcoidosis, lupus, and rheumatoid arthritis, are characterized by inflammation in or near the muscle (8).

1.2.3.2. Toxic myopathy

Toxic myopathy develops when a toxin, medication, or treatment impairs muscle structure or function (9).

1.2.3.3. Endocrine myopathy

Disorder results when hormones interfere with muscle function. Problems with the thyroid or adrenal glands are the most widely known reasons (10).

1.3. Statins

In industrialized world, the rate-limiting enzyme in cholesterol manufacture, statins are among the most commonly prescribed drugs because they lower the risk of coronary artery disease in at-risk individuals (11–13). Seven statins are presently available on the market. The FDA initially approved lovastatin in the US in 1987, then Simvastatin in 1988, Pravastatin in 1991, Fluvastatin in 1994, Atorvastatin in 1996, Rosuvastatin in 2003, and Pitavastatin in 2009 (14,15). Another drug, cerivastatin, was introduced in 1998 but was later taken off the market because of a significantly higher incidence of muscle toxicity. Statins are HMG-CoA reductase inhibitors that have been created to treat hypercholesterolemia. They are the best treatments available for reducing plasma cholesterol, and their high level of tolerance is also valued. Studies have shown that these substances slow the development of atherosclerosis and may even promote its regression. Numerous clinical investigations showed that these effects led to significantly lower cardiovascular morbidity and mortality (16). The ability of HMG-CoA reductase inhibitors to lower endogenous cholesterol synthesis by competitively inhibiting the key enzyme involved is usually cited as one of its favorable effects (17). Inhibition of this important enzyme may have pleiotropic effects since mevalonate, the end

product of the HMG CoA reductase process, serves as a precursor for not just cholesterol but also for a number of other non-steroidal isoprenoidic substances. Statins regulate a number of processes that result in a decrease in the accumulation of esterified cholesterol in macrophages, an increase in endothelial nitric oxide synthetase, a reduction in inflammation, increased stability of the atherosclerotic plaques, and a restoration of platelet activity as well as the coagulation process (18).

1.3.1. Classification of statins

Statins are classified for several types, which includes:

1.3.1.1. Source of statin

Some statins, including lovastatin, pravastatin, and simvastatin, are manufactured synthetically, whereas others, like fluvastatin, atorvastatin, and cerivastatin, are obtained through fungal fermentation (19).

1.3.1.2. Liver metabolism

Liver is the target organ for statin. For fluvastatin, lovastatin, and simvastatin, the liver engaged more than 70% of the dose; for pravastatin, it engaged more than 80%; and for fluvastatin less than 46% (19). When it comes to liver metabolism, fluvastatin uses the CYP 2C9 pathway while lovastatin, simvastatin, atorvastatin, and cerivastatin use the cytochrome P450 (CYP 3A4) pathway. Pravastatin is primarily metabolized by the presence of the glucuronidation reaction with minimal involvement of the CYP 3A enzyme (20) .

1.3.1.3. Solubility

Based on how soluble they are, statins can be categorized into two groups: hydrophilic and lipophilic (21) .Simvastatin, Fluvastatin, Lovastatin, Pitavastatin, and Atorvastatin are primarily lipophilic statins that can simply enter the membranes more deeply where they interact with the surrounding acyl chains. Contrarily, in order to inhibit the HMG-CoA reductase enzyme, hydrophilic drugs (such as

pravastatin and, to a lesser extent, rosuvastatin), require to enter the cell through protein transporters (21). Lipophilic statins can enter cells and diffuse widely throughout many tissues by passive diffusion. However, the liver-specific carrier-mediated pathways necessary for hydrophilic statin absorption may restrict their efficacy in regions beyond the liver (22,23) .

1.3.1.4. Specific activity

Atorvastatin, Cerivastatin, Fluvastatin, and Pravastatin are given as active molecules, but Lovestatin and Simvastatin are given as inactive forms (lactone), which must be enzymatically hydrolized to produce active forms (acid form) (24).

1.4. Atorvastatin

Dr. Bruce Roth discovered atorvastatin for the first time in 1985, and the FDA approved it in 1996 (25). It is a statin drug used to treat abnormal cholesterol levels and prevent cardiovascular disease in people at high risk (26). Statins, which are taken orally, are a first-line medication for preventing cardiovascular disease (26). Atorvastatin and other statins containing lovastatin, pravastatin, rosuvastatin, fluvastatin, and simvastatin are first-line therapies for dyslipidemia (27,28). The growth in cardiovascular diseases (CVD) in many nations is the main cause of the rising use of these medications (29,30). In contrast to other statins, atorvastatin is an active substance and does not need to be activated (31).

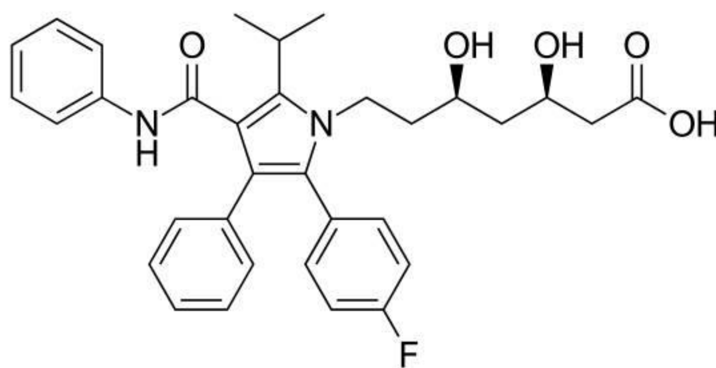


Figure (1-1): Structure of atorvastatin (32).

1.4.1. Mechanism of action

The early rate-limiting step in the formation of cholesterol is the conversion of HMG-CoA to mevalonate, which is catalyzed by the enzyme HMG-CoA reductase, is competitively inhibited by the drug atorvastatin (33,34). Atorvastatin primarily affects the liver, where lower liver cholesterol levels trigger the activation of hepatic low-density lipoprotein (LDL) receptors, resulting in an increase in LDL absorption. Additionally, it decreases the levels of apolipoprotein B (Apo B)-containing particles, serum triglycerides (TG), intermediate density lipoproteins (IDL), very low density lipoprotein cholesterol (VLDL-C), while increasing high-density lipoprotein cholesterol (HDL-C) as shown in figure (1-2) (35).

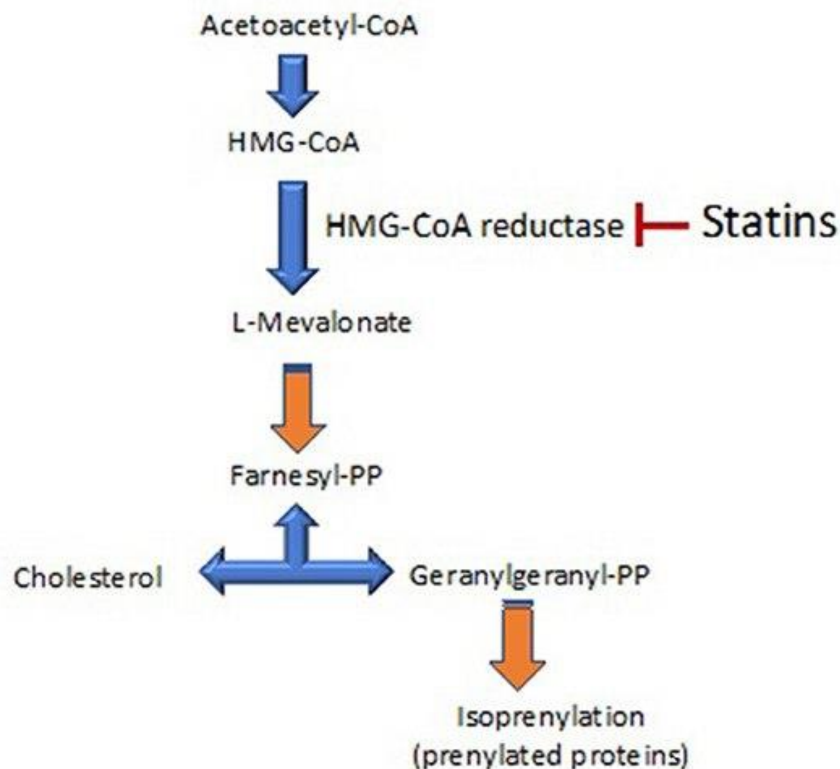


Figure (1-2): The mevalonate pathway (36).

1.4.2. Pharmacodynamics

The liver is the main location for both cholesterol production and LDL removal, making it the most significant location of atorvastatin activity. The amount of LDL-C lowering is correlated with the atorvastatin dose (37). The dose-related magnitude of atorvastatin's impact on blood lipids was established in a Cochrane comprehensive review. Over the 10 to 80 mg/day dosing range, total cholesterol was reduced, LDL cholesterol was reduced by 37.1% to 51.7%, and triglycerides were reduced by 18.0% to 28.3% (38).

1.4.3. Pharmacokinetics

When taken orally, atorvastatin quickly absorbed, taking 1-2 hours to reach its maximum plasma concentration (Tmax) (39) . It has a poor systemic availability mostly due to rapid intestinal clearance and first-pass metabolism (40). Atorvastatin's typical distribution volume is 381 L. It is almost entirely protein-bound (98%) and studies suggest that it is probably released into human breast milk (41). Atorvastatin is extensively metabolized, predominantly by CYP 3A4 in the liver and gut, producing ortho- and parahydroxylated derivatives and other betaoxidation products (41). The enzymes UDP-glucuronosyltransferase 1A1 and 1A3 create acyl glucuronide intermediates, which are then used to further lactonize the metabolites of atorvastatin. The corresponding acid versions of these lactones can be hydrolyzed back to them and exist in equilibrium (42). In vitro, atorvastatin and ortho- and parahydroxylated metabolites both inhibited HMG-CoA reductase. About 70% of the circulating HMG-CoA reductase inhibitory activity is attributed to active metabolites. The majority of atorvastatin and its metabolites are eliminated in the bile in the absence of enterohepatic recirculation. A very small amount of atorvastatin is removed through the kidneys, less than 1% of the total dose (41) .

Compared to its metabolites, which can have half lifes of up to 30 hours, it has a half life of 14 hours (41). 625 ml/min is the total plasma clearance as measured (40).

1.4.4. Medicinal uses

Low density lipoprotein (LDL), Apoprotein (Apo)-B, and triglyceride levels are all reduced by the usage of atorvastatin. Apo B is a cardiovascular risk indicator and LDL is frequently referred to as "bad" cholesterol (43).

Additionally increasing levels of HDL cholesterol (the "good cholesterol"), atorvastatin contributes in the prevention of cardiovascular illnesses. It is also approved for the treatment of dysbetalipoproteinemia, a rare inherited disorder associated with high levels of triglycerides and cholesterol (44).

1.4.5. Adverse effects

Statins are generally well tolerated. The most important adverse effects are liver and muscle toxicity. Myopathy can happen if inhibitors of cytochrom P450 or other inhibitors of statins metabolism are administered together with statins, determining the increase of their blood con- centration. Such are the azole antifungals (45). Fibrates and niacin enhance myopathy risk by a mechanism not involving the increased statins blood concentration. Other risk factors are: hepatic dysfunction, renal insufficiency, hypothyroidism, advanced age and serious infections (46).

1.5. Statin-induced myopathy

The statin-induced myopathy can manifest itself in a variety of ways, ranging from mild myalgia to an extremely rare, potentially fatal form of rhabdomyolysis that causes severe muscular atrophy and renal failure. Even though generalized muscular pains are usually not life threatening, they lower the patients' quality of life (47). Myopathy is the term for muscle pain, discomfort, or weakening brought on by unusually high levels of creatine kinase (10 times the upper limit of normal) (48), unlike the American College of Cardiology/American Heart Association/National Heart, Lung, and Blood Institute (ACC/AHA/NHLBI) task group, who use the word "myopathy" as a catch-all for muscle disorders (49). The incidence of dose-dependent myotoxic symptoms, from mild myalgia to rhabdomyolysis, varies depending on the criteria used, and ranges from 1 to 7 percent (50). Musculoskeletal problems account for ten to fourteen percent of statin-related adverse events reported to the International Drug Information System. Myalgia is the most often reported myotoxic event, accounting for 6 to 14% of statin-related side effects (50), even if the actual frequency may be lower. Muscular symptoms may be falsely attributed to statin therapy even though they are reasonably common, especially in elderly patients, if the patient is aware that therapy-related muscle problems are widespread (51).

Table(1-1): The clinical spectrum of statin-induced myopathy (52).

Condition	Definition
Myopathy	All negative effects connected to skeletal muscle are together referred to as myopathy.
Myalgia	weakness or discomfort in the muscles but no increase in CK.
Myositis	Symptoms of muscle with elevated CK are usual.
Rhabdomyolysis	Muscle aches and pains associated with CK increase (generally >10 ULN) and elevated creatinine.

1.5.1. Epidemiology

The likelihood of statin-related muscle complaints varies by statin class, with lipophilic statins like atorvastatin carrying the highest risk due to their tendency to nonselectively diffuse into extra hepatic tissues like skeletal muscle (53). There have been reports of various statin myopathy frequencies. Rarely does rhabdomyolysis happen. However, less serious side effects occur more commonly. For instance, between 2 and 10.5 percent of the time, myalgia is recorded. These different percentages are most likely a result of unequal assessment severity, which includes different definitions, assessment methods, and reporting biases (e.g. drug reporting systems, awareness and publicity). In addition, the majority of statin clinical trials did not include a specific evaluation of muscle-related disorders as their goal (54). There are very few clinical trials that are large enough and long enough to identify rhabdomyolysis (55) .

1.5.2. Pathophysiology

Myopathy brought on by statins is caused by a complex, multifaceted mechanism that is poorly understood. Statin-induced myopathy is thought to be caused by the apoptosis of skeletal muscle cells, which is triggered by disrupted intracellular calcium signaling, and mitochondrial dysfunction, which results from the decrease in the production of ubiquinone (coenzyme Q10), a component of the mitochondrial electron transport chain. As a result, ATP synthesis and free radical scavenging are both diminished (56). Reduced ATP synthesis as a result of altered Na+/K+ pump density (57).Changes in the skeletal muscle cell's fluidity and cholesterol content impair the normal function of the muscle (58).

1.6. Risk factors of statin induce myopathy

1.6.1. Gene related risk factors

1.6.1.1. Variants of CYP450

Simvastatin, atorvastatin, and lovastatin all undergo phase I metabolism mostly via the CYP3A4 pathway. The effectiveness and tolerability of statin medication are impacted by the CYP3A4 gene's activity, which can vary up to 10-fold amongst patients. With more than thirty recognized isoenzymes, the cytochrome enzyme system is the primary enzyme system in charge of the phase I metabolism of different statins (59). With the exception of pitavastatin, fluvastatin, and rosuvastatin, practically all statins are affected by phase I oxidation by the CYPs (60). All of the major CYPs, including CYP3A4, CYP3A5, CYP2D6, and CPY2C9, are involved in the metabolism of statins. Genetic variants that can change how CYP2D6, CYP3A4/5, and other proteins function are particularly fascinating. CYP3A4 is the main route for the metabolism of statins. Although CYP2D6 and CYP3A5 are not the primary enzymatic pathways involved, multiple studies have

revealed that their genetic variants may emphasize interindividual variability in connection to response and adverse medication reactions in particular statins (61).

1.6.1.2. Variants of solute carrier organic anion transporter family member 1B1 (SLCO1B1)

Some of the diversity in statin therapy tolerance and outcome may be explained by variations in SLCO1B1 gene expression between people. The gene for the protein known as OATPIB1, also known as the organic anion transporting polypeptide IBI (SLCO1B1), is found on the short arm of chromosome 12. It moves substances from the blood into the liver cells, where it is present, including both endogenous and xenobiotic compounds. Numerous studies have revealed that this transporter is crucial for the uptake of statins (62). Simvastatin, pravastatin, atorvastatin, and rosuvastatin all work through this mechanism (63). Due to its lipophilicity, fluvastatin can easily cross the hepatocyte membrane, or it can do so by employing another transporter (64). A diminished capacity to metabolize these medications is connected to some widespread variations in the SLCO1B1 gene. Reduced-function variants of SLCO1B1 have been identified as risk factors for muscle toxicity and rhabdomyolysis due to the protein's low hepatic influx activity. This results in increased blood concentrations of statins and greater contact with muscle tissues (65).

1.6.1.3. Variants of ABCB1

The ABC superfamily of proteins includes the ABCB1 protein. P-glycoprotein (P-gp), commonly known as multidrug resistance (MDR1), is a 170 kDa glycosylated membrane protein that is expressed in numerous organs, including the liver, intestines, kidney, brain, and testis (66). In these regions, ABCB1 is found on the cell membrane and functions as an efflux transporter to clear cells of metabolites and a variety of hydrophobic foreign compounds, including medicines.

ABCB1 maintains the blood-brain barrier and aids in the removal of medications into the urine or bile due to its placement on certain cells (67).

1.6.1.4. Variants of COQ2

Some COQ2 gene variants that code for parahydroxybenzoate-polyprenyltransferase, another enzyme involved in the mevalonate pathway, are linked to CoQ10 deficiency (68). Energy production is decreased when CoQ10 (ubiquinone), an essential component of the mitochondrial respiratory chain, is lacking. CoQ10 is transported by LDL and HDL because it is produced via the mevalonate pathway and is lipophilic in plasma. By directly inhibiting the mevalonate pathway enzyme HMG-CoA reductase and by reducing CoQ10 transport capacity as a result of lower LDL levels, statins reduce CoQ10 levels in two different ways (69). The frequency of genetic variation in the COQ2 gene between statin-tolerant and tolerant people was investigated in a previous study (52). The second gene in the process that creates CoQ10 is the COQ2 gene. The COQ2 gene's single nucleotide polymorphism (SNP) and statin myopathy were found to be significantly correlated (70) .

1.6.2. Statin-related risk factors

1.6.2.1. Pharmacokinetics

Lipophilicity: pravastatin and rosuvastatin are the most hydrophilic statins and require active transport into the hepatocyte. Transmembrane transport of statins in hepatic cells is actively mediated through organic anion transport polypeptides (OATP) (71). Hydrophilic statins are less likely to enter non-hepatic cells such as myocytes and thus might have less risk of myopathy (72).

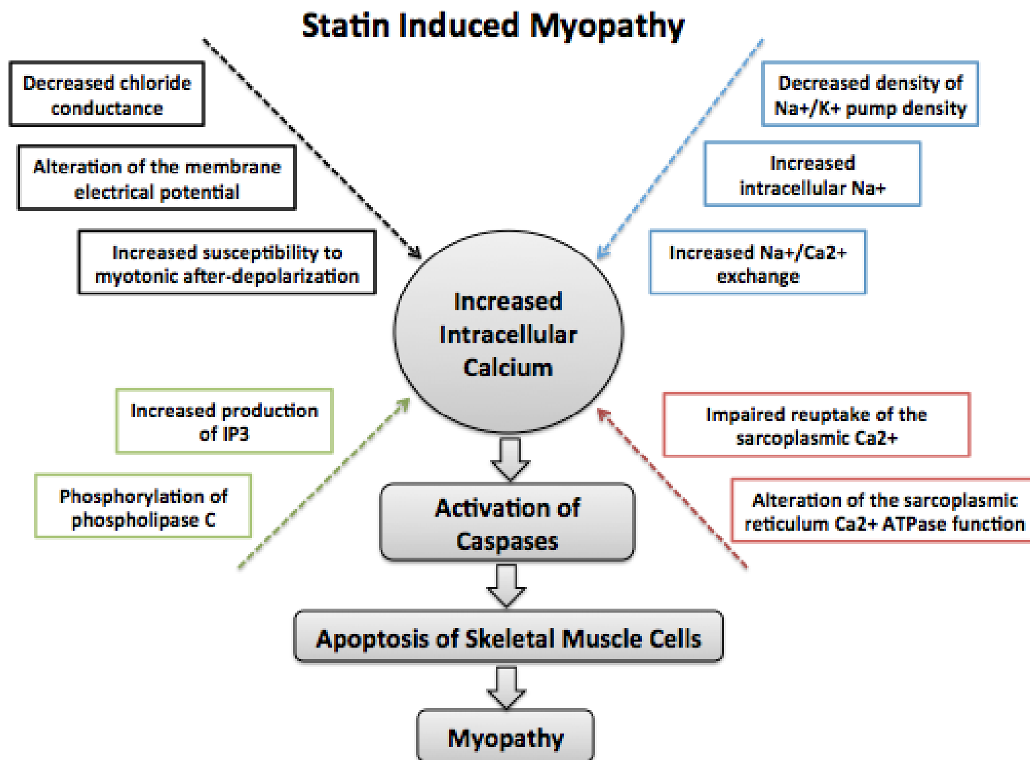
1.6.2.2 Dose of statin

While the achieved LDL decrease is related to the therapeutic advantages of statin (73), The degree of LDL reduction appears to be less important for

myopathy than statin dosage (74). With instance, for dosages of 20, 40, and 80 mg, respectively, myopathy incidence was 0.02, 0.08, and 0.53 percent with increasing simvastatin dosages (75).

1.7. Mechanisms of statin-induced myopathy

Numerous theories have been proposed, however the precise mechanism causing statin-induced myopathy has not yet been identified. As shown in figure(1-3) some of the most likely scenarios are isoprenoid depletion, ubiquinone or



coenzyme Q10 (CoQ10) production inhibition, decreased or altered sarcolemal membrane cholesterol, impaired calcium metabolism, or autoimmune reactions (76).

Figure(1-3) Proposed mechanisms of statin-associated muscle symptoms (76).

1.7.1. Isoprenoid depletion

One suggested mechanism of myopathy is statin-induced isoprenoid deficiency. Isoprenoids are lipids that are by-products of the HMG-CoA reductase pathway (77). Farnesyl pyrophosphate (F-PP) and geranylgeranyl pyrophosphate

(GG-PP) are the important isoprenoids in the HMG-CoA reductase pathway . Isoprenoids are connected to proteins by either farnesylation or geranylgeranylation in a process called protein prenylation. Protein prenylation is a post-translational process during which two types of isoprenoids (F-PP and GG-PP) are added covalently to cysteine residues at or near the C terminal (78). Statins decrease prenylation of proteins and encourage alternative modification (dysprenylation) (79). Two important proteins are affected by dysprenylation, the ‘small GTPases’ and the ‘lamins’. Dysprenylation of small GTPases causes vacuolation of myofibers, degeneration and swelling of organelles and ultimately apoptosis. In addition, dysprenylation of lamins increases cell susceptibility to mechanical stress (79,80). The reduction of protein prenylation (farnesylation or geranylgeranylation) is thought to rise cytosolic calcium which activates capsase-3 and leads to cell death. Supporting the role of isoprenoids in statin myopathy is the finding that statin-induced apoptosis in vascular smooth muscle cells is stopped by supplementation with isoprenoids including F-PP and GG-PP (81).

1.7.2 Inhibition of ubiquinone or CoQ10 production

Ubiquinone or CoQ10 is essential of oxidative phosphorylation and ATP production in the mitochondria (82). Statins prevent the synthesis of mevalonate, which is a CoQ10 precursor. Theoretically, statins can cause myopathy by inhibiting the synthesis of CoQ10 in the mitochondria which might compromise the function of the mitochondrial respiratory chain, impair energy production and eventually induce myopathy (83). Statins lower serum levels of CoQ10 (84). However, it is not known if this decrease is due to a decrease in LDL-C which transports CoQ10 or due to drug-induced inhibition of CoQ10 synthesis (85).

1.7.3 Decreased sarcolemal cholesterol

Myocyte membrane cholesterol changes as cholesterol levels fall (86). The dynamic balance of plasma and membrane lipids results in sarcolemal cholesterol shortage may negatively alter the integrity and fluidity of the membrane, leading to membrane instability (87). However, two key findings argue against this mechanism, the first one is that myotoxicity does not occur in vitro when cholesterol is lowered by inhibiting squalene synthetase in human skeletal myotubules (88). The second finding is that inherited disorders of the distal cholesterol synthetic pathway result in decrease cholesterol levels without associated clinical myopathy (89).

1.7.4. Disturbed calcium homeostasis

The regulation of calcium (Ca^{+2}) release and uptake is important for the regular function of muscle cells. The initial increase in the intracellular Ca^{+2} by the action potential is mediated by L-type Ca^{+2} channels. This increase in the intracellular Ca^{+2} opens the ryanodine receptors located in the sarcoplasmic reticulum causing a noticeable increase in the intracellular Ca^{+2} to initiate the muscle contraction. In a previous study, muscle biopsies from patients with statin myopathy showed increased expression of ryanodine receptors 3 (RR3). Whether increased RR3 is a risk factor of statin myopathy or a result of it is still to be determined (90).

1.7.5. Autoimmune phenomena

Autoimmune mechanisms for statin myopathy is supported by the fact that certain autoimmune diseases such as polymyositis, lupus and myasthenia gravis can be worsen by statins. Statins also have been shown to cause MHC-II-dependent activation of T lymphocytes (91,92). In previous study eight cases of statin-induced myopathy in whom symptoms persisted or progressed after stopping statins (93). The study suggested a mechanism by which statins induce upregulation of MHC-I expression and antigen presentation by muscle fibers (94). (96). Statins have also

been reported to cause severe immune-mediated necrotizing myopathy that needs treatment with immunosuppressive therapy (95,96) .

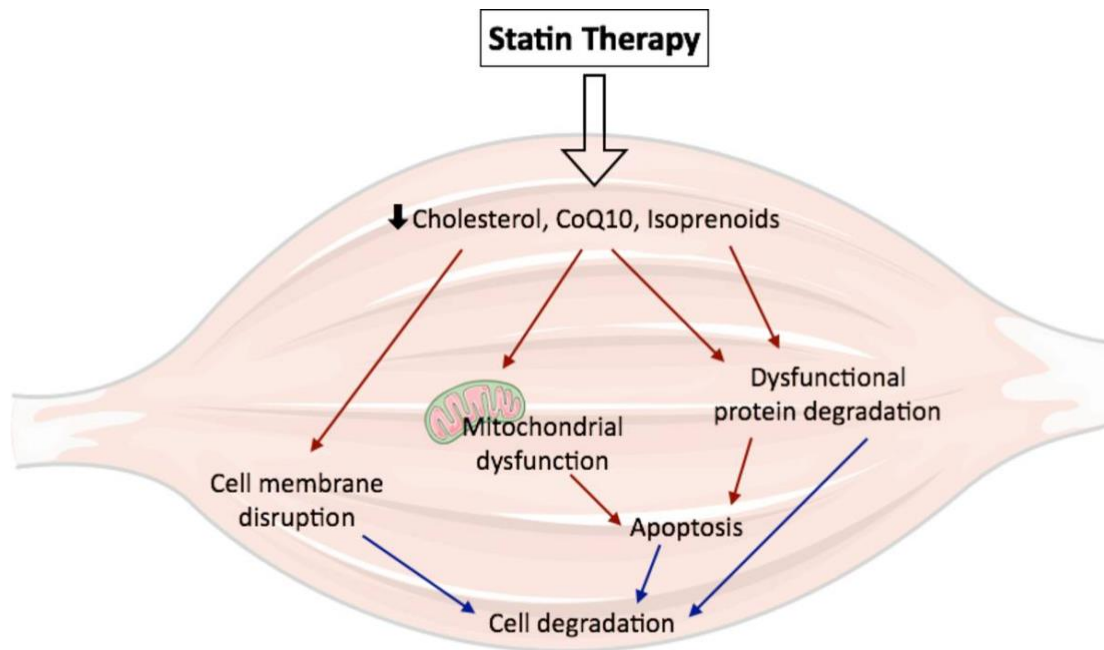


Figure (1-4): Current theories of the possible mechanisms contributing to statin myopathy (97).

1.8. Diagnosis

1.8.1. History and Symptoms

There is a continuum of statin-induced myopathy symptoms, from moderate and asymptomatic to severe and fatal. Although the median period for the onset of symptoms is four weeks after the start of treatment, the exact timing varies from person to person. The length of time it takes for symptoms to go away after proper care also differs between people (98) .The patient's medical history should include information regarding the patient's medications, past trauma, and a description of the nature of the muscle discomfort.

1.8.2. Laboratory Tests

Creatine kinase should be measured as part of the diagnostic evaluation when a patient exhibits symptoms suggestive of statin-induced myopathy. To rule

out other illnesses that can cause myalgia, it is necessary to obtain the TSH levels (99).

1.9. Role of genetic polymorphisms in drug response

Pharmacogenomics and pharmacogenetics work to define how genetic variables affect the effectiveness of medications and adverse drug effects. Pharmacogenomics attempts to simultaneously examine many genes and their interactions, in contrast to pharmacogenetics, which is focused on the pharmacological effects of a single gene mutation (100). A main progress in pharmacogenetics/pharmacogenomics has been possible thanks to the genetic revolution with the human genome project and the development of modern technologies in genetic testing. It has been estimated that personalized medicine considering patients' individual genetic profile would soon be introduced into clinical practice. Since variation in drug concentrations resulting from respective genetic polymorphisms in drug metabolizing enzymes could be directly implemented into dose adjustments, genotyping for drug metabolizing enzyme DMEs seems to be the closest to incorporation into clinical practice (101). On the other hand, genotyping for drug transporters and receptors is not yet suitable for therapeutic use, so it cannot be suggested as a method for enhancing drug therapy in clinical practice. The importance of genetic variants in DMEs in explaining interindividual variability in drug concentrations and associated pharmacodynamic effects was first recognized by researchers over 50 years ago (102) .Early examples include the metabolization of sparteine, isoniazid, debrisoquine, or succinylcholine. The functional implications of genetic polymorphisms have already been investigated for the majority of DMEs. There have been reports of variations with fully inactive enzymes as well as those with active enzymes that are more or less active than the wild-type alleles. Thus, for a fraction of drugs, it would be desirable

to consider individual activity of respective DMEs as the basis for optimizing therapy (103).

1.9.1. Coenzyme Q2 Gene

COQ2 (p-hydroxybenzoate polyprenyl transferase) encodes the enzyme required for the second stage of the final reaction sequence for the biosynthesis of Coenzyme Q10 (CoQ10). This enzyme catalyzes the second step of the final reaction series of CoQ biosynthesis, the condensation of 4-hydroxybenzoate with polyprenyl-pyrophosphate, producing the first membrane-bound CoQ intermediate (104).

COQ2 gene is positioned at 4q21 and has 7 exons. The encoded protein is parahydroxybenzoate polyprenyl transferase, which catalyzes the prenylation of parahydroxybenzoate with a polyprenyl group. Human COQ2 protein contains nine transmembrane domains, a cytoplasmic domain, and a non-cytoplasmic domain. The activity of the enzyme is primarily in the transmembrane domain and the non-cytoplasmic domain (105).

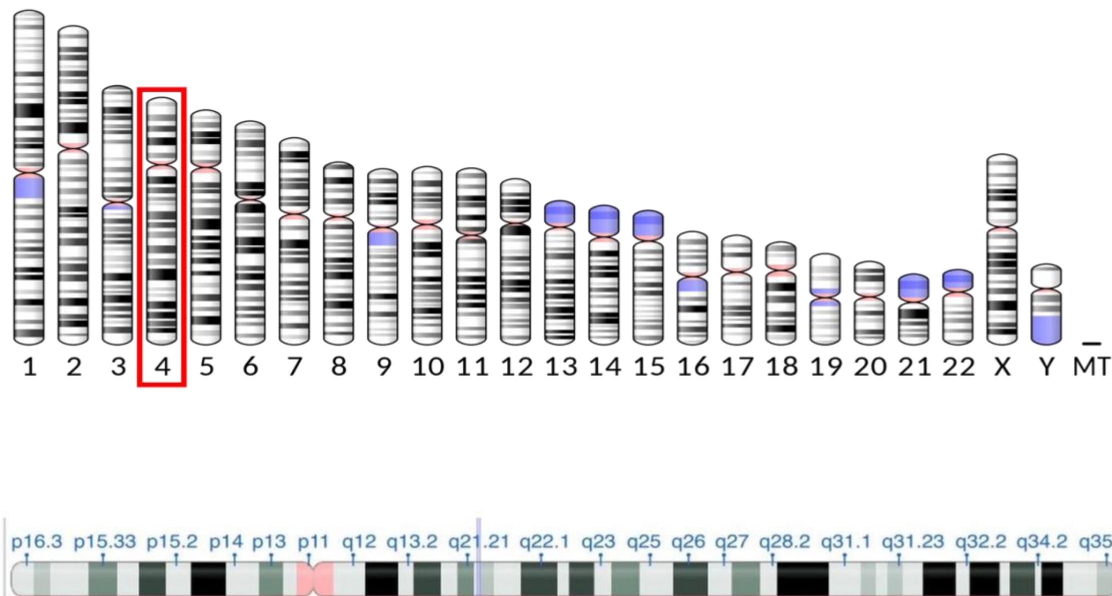


Figure (1-5): Location of COQ2 gene(106).

1.9.1.1. Normal Function

The COQ2 gene (polyprenyltransferase), provides instructions for making an enzyme that carries out one step in the production of a molecule called coenzyme Q10, which has numerous critical functions in cells throughout the body. In cell structures called mitochondria, coenzyme Q10 plays an important role in a process called oxidative phosphorylation, which converts the energy from food into a form that cells can use (107) .Coenzyme Q10 is also involved in producing pyrimidines, which are building blocks of DNA, RNA, and molecules such as ATP and GTP that serve as energy sources in the cell. In cell membranes, coenzyme Q10 acts as an antioxidant, protective cells from damage caused by unstable oxygen containing molecules (free radicals), which are byproducts of energy production(108).

1.9.1.2. Health Conditions Related to Genetic Changes

1.9.1.2. A. Primary coenzyme Q10 deficiency

At least nine mutations in the COQ2 gene have been connected to a syndrome known as primary coenzyme Q10 deficiency. Although it can happen at any age, this uncommon condition typically manifests in early childhood or infancy. It can affect many different body organs, but most frequently the kidneys, muscles, and brain (109) .The COQ2 gene mutations associated with this disorder greatly decrease or eliminate the production of the COQ2 enzyme, which prevents the normal production of coenzyme Q10. Studies suggest that a deficiency of coenzyme Q10 impairs oxidative phosphorylation and increases the susceptibility of cells to damage from free radicals (110). A deficiency of coenzyme Q10 may also disrupt the production of pyrimidines. These changes can cause cells through the body to malfunction, which may help explain the variety of organs and tissues that can be affected by primary coenzyme Q10 deficiency (111).

1.9.2. Coenzyme Q10

Coenzyme Q10 was discovered by Crane in 1957 (112). Coenzyme Q10 is a quinone that is naturally fat-soluble and can be found in hydrophobic sections of cellular membranes. The body produces the remaining half of its own CoQ10, with around half coming from dietary fat (113).

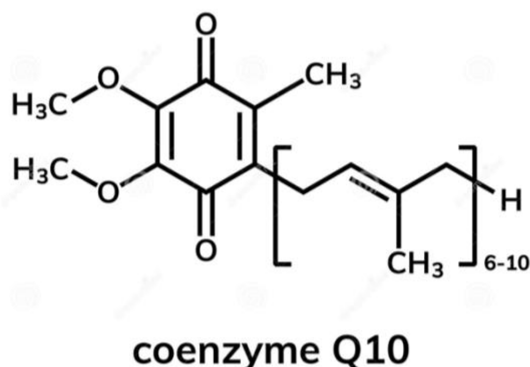


Figure (1-6): structure of coenzyme Q10 (114).

Coenzyme Q10 protects against oxidative stress brought on by free radicals and aids in the transfer of electrons during mitochondrial oxidative phosphorylation (115) , and regenerates the antioxidants tocopherol (vitamin E) and ascorbic acid in their active forms (116). Farnesyl pyrophosphate, a step in the synthesis of CoQ10, is blocked by statins. This information, along with the significance of mitochondria in generating energy for the muscles, has led to the theory that statin-induced CoQ10 depletion leads to statin-associated myopathy (117).

1.9.2.1. Biosynthesis of COQ10

The de novo production of CoQ10 takes place mostly in the mitochondria, where it plays a crucial role in the mitochondrial respiratory chain. Human CoQ10 production is a complicated biological process that is still not fully understood (118). As a result, the process of its manufacture in different organisms, such as yeasts and bacteria, has been identified. CoQ10 is composed of a benzoquinone ring and a

polyprenyl side chain. Tyrosine or phenylalanine is used to make the benzoquinone ring, while intermediates from the mevalonate pathway are used to make the polyprenyl side chain (118).

Decaprenyl diphosphate (decap renyl-PP) is produced from mevalonate by the PDSS1-PDSS2 enzyme complex via the intermediates farnesyl-PP and geranylgeranyl-PP, as shown in Figure (1.7). Parahydroxybenzoate-polyprenyl transferase, also known as COQ2, then catalyzes the condensation of decaprenyl-PP with para-hydroxybenzoate, which is created from tyrosine or phenylalanine. At least eight more COQ enzymes (COQ3-COQ10A, B), which catalyze the methylation, decarboxylation, and hydroxylation activities, are required to produce functional CoQ10 (119).

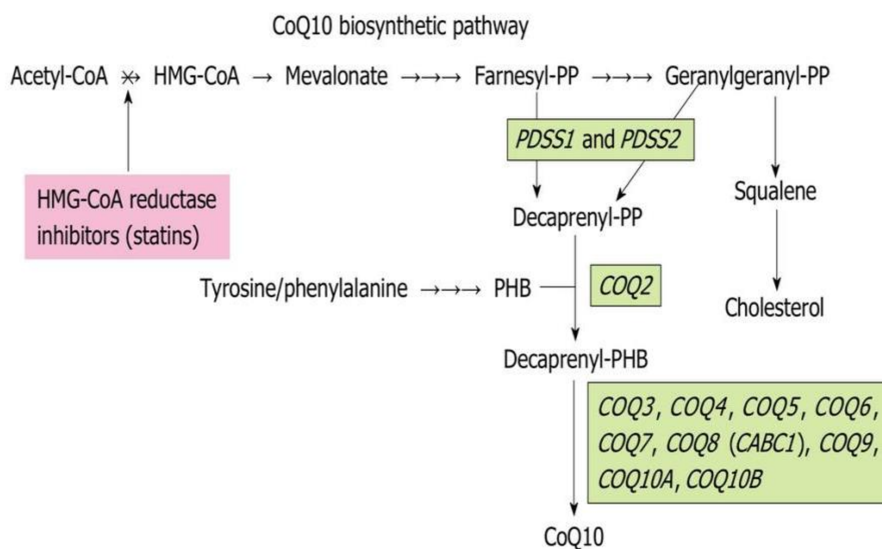


Figure (1-7): Coenzyme Q10 production pathway. Italicized symbols for human genes and enzymes are displayed. HMG-CoA stands for 3-hydroxy-3-methylglutaryl coenzyme A, PP stands for pyrophosphate, PHB stands for para-hydroxy-benzoate, CoQ10 stands for coenzyme Q10, and CABC stands for chaperone-activity of bcl (120).

1.9.2.2. Effect of statin on CoQ10

Despite the fact that statins have been shown to affect circulating CoQ10 levels in people, low-dose statin therapy does not appear to reduce intramuscular CoQ10 levels (70). Mixed outcomes have been seen in studies employing muscle biopsy samples from patients with myopathy brought on by statins. For instance, despite a moderately decreased amount of muscle CoQ10, another study revealed no biochemical or histochemical evidence of mitochondrial myopathy despite morphological alterations that are thought to be associated with mitochondrial failure (121). Supplemental CoQ10 can raise levels in the blood, but it's unclear if this will help with muscle pain (122).

1.10. Prevention of statin-induced myopathy

All statin-treated patients who complain of muscle problems should have their CK levels tested as well as the severity of their symptoms assessed. Other reasons of muscle discomfort, such as strenuous activity, hypothyroidism, or alcohol abuse, should be ruled out by medical authorities (123). Patients should be assessed for rhabdomyolysis and the need to stop taking statin medication if their CK level rises to more than 10 times the upper limit of normal. The choice to continue or stop taking statin therapy for people with normal or mildly elevated CK (less than five times ULN) depends on how well they tolerate their symptoms. A normal CK does not entirely rule out structural muscle injury because this can happen even in the absence of a CK increase (124). Patients should discontinue taking statins if their symptoms become intolerable. After ruling out other potential reasons, stopping statin therapy is the cornerstone of addressing myalgia brought on by statin use. Some have recommended CoQ10 for the prevention and treatment of statin myopathy.Contradictory results have been seen in clinical studies, and there is no relationship between intramuscular and plasma levels of CoQ10. The national lipid

association (NLA) does not support CoQ10 therapy as a result (125). Nevertheless, one study on the effects of CoQ10 came to the conclusion that its use can be advised for patients who experience statin myalgia and are unable to adequately treat themselves with any other medications because there are no known dangers for CoQ10 supplements (69).

1.11 Aim of the study:

The aim of this study is to detect the role of genetic polymorphism of COQ2 gene particularly COQ2 (A>G) (rs6535454) and COQ2 (C>A) (rs6818847) that involved in the incidence of myopathy in Iraqi patients treated with 40mg atorvastatin.

CHAPTER TWO

PATIENTS, MATERIALS AND METHOD

2.1 Materials

2.1.1 Instruments

The instruments that used in this study with their manufacture and origin are listed in table (2-1).

Table (2-1): Instruments used in this study with their manufacture and origin.

Instrument	**Manufacture/Origin**
Autoclave	LabTek/ Korea
Balance	Precis / Switzerland
Digital camera	Canon / England
Distillator	GFL / Germany
Electrophoresis apparatus	Consort / Belgium
High speed centrifuge	Mikro 200R Hettich / Germany
Hood	LabTech / Korea
Hoot plate stirrer	LabTech DAIHAN / Korea
Micro - Centrifuge	BIONEER / Korea
Micropipettes	Eppendorf / Germany
Nano drop	Bio Drop / England
Refrigerator	Concord / Lebanon
Sensitive balance	AND/ Taiwan
Thermos cycler (PCR apparatus)	Cleaver / USA
UV trans illuminator	UV-VIS /JAPAN
Vortex	Human Twist / Germany
Water bath	LabTech / Korea

2.1.2 Chemicals and Kits

Specific chemicals and kits that used in this study are listed in table (2-2.)

Table (2-2): Kits and chemicals used in this investigation, together with information on where they were made.

Kits and chemicals	Manufacture/Origin
Absolute ethanol	Systerm/ Malaysia
Agarose	BDH / England
DNA ladder	Bioneer / Korea
DNA extraction kit	Favorgen Bio Tech /China
Ethidium Bromide	Sigma / USA
Human coenzyme Q10 Elisa kit	BT LAB / China
Nuclease free water	Promega / USA
PCR Master Mix	Promega/USA
Primers	Bioneer / Korea
TBE (Trise Borate EDTA) buffer solution	Bioneer / Korea

2.2 Patients

2.2.1 Study population

The Imam Al-Hussein Medical City/Cardiology Center and Al-zahraa Center in Kerbala conducted this cross-sectional observational study from October 2021 to February 2022. The Scientific and Ethical Committee of Pharmacy College/Kerbala University accepted the study's protocol, and each subject signed an informed consent form after being told of the study's nature and objectives. 150

individuals, ranging in age from 30 to 65, participated in the trial and took 40 mg of atorvastatin orally each day.

2.2.1.1 Inclusion Criteria

All patients (male and female) who had been taking atorvastatin for at least one month prior to the study's start and who were free of any major diseases were required to meet the inclusion criteria.

2.2.1.2 Exclusion Criteria

1. Patients with severe renal and liver impairment.
2. Multiple medication.
3. Advanced age.
4. Low body mass index.

2.2.2. Collecting clinical data

Each patient was asked if they had taken any medications that might affect how atorvastatin is metabolized when blood samples were being taken. This was done to ensure that all potentially interfering medications were recorded in the database. Data on age, weight, height, education, smoking, type of myopathy, length of treatment, other drug side effects, other diseases, and any other drugs used were gathered from the medical records of willing patients as well as directly from the patients.

2.2.3 Sample collection and analysis

Blood samples were obtained from qualified patients who had completed informed permission forms and were approved by the Scientific and Ethical Committee of the Pharmacy College/Kerbala University. Each patient who participated in this trial had five ml of venous blood taken. The EDTA tube was filled with two milliliters of blood for molecular analysis. Three ml was placed in a gel tube then centrifuging at 3000 rpm for 20 minutes,the serum was obtained, and

it was used to measure thyroid-stimulating hormone, creatinine levels, creatine kinase, Coenzyme Q10 and the lipid profile (cholesterol, triglycerides, low density lipoprotein, and high density lipoprotein).

2.3 Methods

2.3.1 Molecular analysis

2.3.1.1 DNA Extraction

According to the procedure for the favorgen for blood genomic DNA extraction kit, genomic DNA was extracted from a blood sample.

For DNA extraction from blood,the following technique is suitable:

1. 300 µl of blood transfer to a 1.5 ml eppendrof tube, then 900 µl of RBC lysis solution was added. The mixture was incubate at room temp for 10 min.
2. The eppendrof tube was centrifuged at 3,000 x g for 5 minute, the supernatant was removed except the white cell pellet and only about 50-100 µl of the remnant was remained.
3. 100 µl of cell lysis solution was added to the re-suspended cells and then pipetting up and down was done to lyse the cells.
4. 10 µl of proteinase k was added to cell lysate then vortexed vigorously at high speed for seconds. Then 200 µl of FABG buffer was added to the mixture.
5. The tube was vortexed strongly and then the sample mixture was incubated at room temperature for 10 min and during the incubation, the tube was inverted every 3 min.
6. 200 µl of 100% ethanol was added to the sample and vortexed for 10 seconds.
7. FABG column was placed to the collection tube.
8. The sample mixture was transferred carefully to FABG column and centrifuged it at speed 14,000 rpm for 1 min.

9. The mixture in the collection tube was discarded and the FABG column was placed to a new collection tube.
10. 400 µl of W1 buffer was added to the FABG column and then centrifuged at speed 14,000 rpm for 1 min.
11. The flow through was discarded and then the FABG column was placed back again to the collection tube.
12. 600 µl of wash buffer was added to the fABG column and then centrifuged it at speed 14,000 rpm for 1 min.
13. The flow through was discarded and then the FABG column was placed back again to the collection tube.
14. The dry FABG column was placed to anew eppindrof tube.
15. 100 µl of preheated elution buffer was added to the membrane center of FABG column and then centrifuged it at speed 14,000 rpm for 1 min to elute the DNA.
16. The DNA was collected and long term stored at -20 °C (deep freezing).

2.3.1.3 Polymerase Chain Reaction

Polymerase chain reaction is the process that involve DNA regions replication through using an enzyme DNA polymerase which permits amplification of desired DNA fragments from one molecules to several million copies; many requirements need to proceed PCR reaction such as two primers which are complementary to the target DNA one primer bind to each side of DNA and between them the desired DNA sequences will be amplified. There are three main steps for any PCR that are cycled about 25-45 times, which include:

A. Denaturation: this step occurs at 94-95°C and requires decoding of double strand DNA into single strands.

B. Annealing: this step occurs at 55-65°C, the reaction begins by stiffen the pair of short oligonucleotide sequences to the ends of the strands of DNA template.

C. Extension: this step occur at 72-74°C, it needs extension of the primers to form new strand complementary to the template table and this happen in the presence of DNA Taq polymeras (126).

There are several polymerase chain reactions (PCR) techniques differ in the principle, in this study use the technique:

Amplification refractory mutation system (ARMS-PCR): refers to mutation detection method based on specific PCR primers and also called amplification of specific alleles, in which specific set of primer; two forward primers (forward wild and forward mutant type) and two reveres primers which are complementary to the DNA template which include the region to be amplified (127).

2.3.1.4 Primers design

The COQ2 gene (C>A) (rs6818847) and (A>G) (rs6535454) specific primer pairs were built using Primer blast software, which is based on the https://www.ncbi.nlm.nih.gov/ websites, to perform polymerase chain reactions. The primer sequences that were used for the COQ2 gene's amplification study to identify SNPs are displayed in Tables (2.3) and (2.4), respectively.

Table (2.3) : lists the COQ2 (rs6818847) (C>A) genetic polymorphism's primer Sequences**.**

SNPs	Primer sequence(5 '->3 ')	Product size	Reference
O-F	CCGCATGAGGCGCAAGTACGGC	474 bp	Current study
O-R	TTGCATCCTGCGGGTGCCACTG		Current study
I-F allele C	CCGGCAGCCACGCCAGTGCTAC	146 bp	Current study
I-R allele A	CGCGGGGCCTGCGGGTTT	204 bp	Current study

Table (2-4): COQ2 (A>G) (rs6535454) genetic polymorphism primer sequences.

SNPs	Primer sequence(5 '->3 ')	Product size	References
IF	TTTCTTTACCTGATGGGCATAAATATTA	211 bp	Current study
IR	CTGGAGTTATGTGGACACTAATATATTAC	166 bp	Current study
OF	TGGTTCTTAAAGTTCTTAAAAAACAACA	320 bp	Current study
OR	ACTCCTTCCCTTTAGGATTCTAAAGT		Current study

A particular amount of nuclease-free water was used to dissolve lyophilized primers to produce a primary concentration of (100 Pmol/ µL) (as stock solution).

Tables (2.5) and (2.6) show how much nuclease-free water is needed for each primer to produce 100 Pmol/L.

Tables (2-5):The Volume of nuclease-free water that was added to each primer of COQ2 (C>A) (rs6818847) to achieve 100 Pmol/ µL..

Primers of COQ2 (rs6818847)	**Volume of nuclease free water added (µL)**
O-F	250
O-R	250
I-F allele C	250
I-R allele A	250

Table (2–6) :the volume of nuclease-free water that was added to each COQ2 (A>G) (rs6535454) primer to produce 100 Pmol/ µL.

Primers of COQ2 rs6535454	Volume of nuclease free water added (µL)
O-F	250
O-R	250
I-F allele A	250
I-R allele G	250

Working solution (10 pmol/L) was created by diluting 10 ul of stock solution with 90 ul of nuclease-free water. The primer was then stored at -20 °C until further usage.

2.3.1.5. Polymerase chain reaction optimization conditions

Optimization of polymerase chain reaction was attained after several trails.

2.3.1.5.1 Optimization of COQ2 (C>A) (rs6818847) genetic polymorphism conditions.

COQ2 (C>A) (rs6818847) optimization of PCR conditions was prepared by using:

- ✓ Different volumes of primers (1µL, o.5 µL)
- ✓ Different annealing temperatures (55.7°C,65°C)
- ✓ Different volumes of DNA sample (3 µL,5 µL)

The best results of this SNP was obtained in the following conditions:

A. 1 µl outer forward primer
B. 1 µl outer reverse primer
C. 1 µl inner reverse primer

D. 1 µl inner forward primer

E. 3 µl DNA sample

F. 12 µl Master mix

G. 6 µl nuclease free water

2.4.1.5.2 Optimization of COQ2 (A>G) (rs6535454) genetic polymorphism conditions.

COQ2 (A>G) (rs6535454) optimization of PCR conditions was prepared by using:

- ✓ Different volumes of primers (1 µL.1.2 µL)
- ✓ Different annealing temperatures (55.7°C, 60°C)
- ✓ Different volumes of DNA sample (3 µL, 5 µL)

The best results of this SNP was obtained in the following conditions:

A. 1.2 µL outer reverse primer

B. 1.2 µL outer forward primer

C. 1.2 µL inner forward primer

D. 1.2 µL inner reverse primer

E. 3 µL DNA sample

F. 12 µl Master mix

G. 5.2 µL nuclease free water

A total volume of reaction was (25 µL) which centrifuged at 2000 rpm for 10 seconds in a micro centrifuge for mixing the sample tubes and then placed in thermocycler.

2.3.1.6 Running & working solution of PCR

2.3.1.6.1 ARMS-PCR running & working solution

The thermal program for COQ2 (C>A) (rs6818847) demonstrated in table (2.7).

Table (2–7): COQ2 (C>A) (rs6818847) Polymorphism PCR thermocycler protocol.

Steps	Temperature (°C)	Minute: second	Cycles
Initial denaturation	95°	05: 00	1
Denaturation	95°	00:30	35
Annealing	65°	00:35	
Extension	72°	00:55	
Final extension	72°	05: 00	1

The thermal program for COQ2 (C>A) (rs6535454) demonstrated in table (2.8).

Table (2–8): COQ2 (A>G) (rs6535454) Polymorphism PCR thermocycler protocol.

Step	Temperature (°C)	Minute: second	Cycles
Initial denaturation	95°	05:00	1
Denaturation	95°	00:30	35
Annealing	57°	00:35	
Extension	72°	00:55	
Final extension	72°	05:00	1

2.3.1.7 Agarose Gel Electrophoresis

1. Agarose gel was prepared by dissolving 0.5 gm of agarose powder was put in beaker, and then 5mL of 10X TBE buffer (tris- borate-EDTA) and 45 mL of distilled water was added.
2. The mixture was warmed on hot plate, and left for few seconds when the mixture began to boil.
3. The solution was left to cool and 3µL of ethidium bromide was added.
4. The comb was fixed on one end of the try to make holes where the samples were loading.
5. After the agarose solution had poured to try, it has been left to congeal at 25 ℃.
6. The comb was removed lightly away from the try.
7. The try was stabled into the device chamber, and the chamber was filled with 1X TBE buffer.
8. One of the wells of agarose gel was loaded with 5 µl of DNA ladder
9. While the others were loaded with 5 µl of each PCR products.
10. The voltage of the electrophoresis apparatus was adjusted at 45 volts to ensure an electrical field adjusted with (5) v/cm for 10 cm distance between cathode and anode.
11. At the end of the run, ultraviolet treans-illuminator was used for detection of the bands.
12. The gel was photographed using digital camera (canon EOS 80 D).

2.4. Biochemical parameters

2.4.1 Determination of Coenzyme Q10

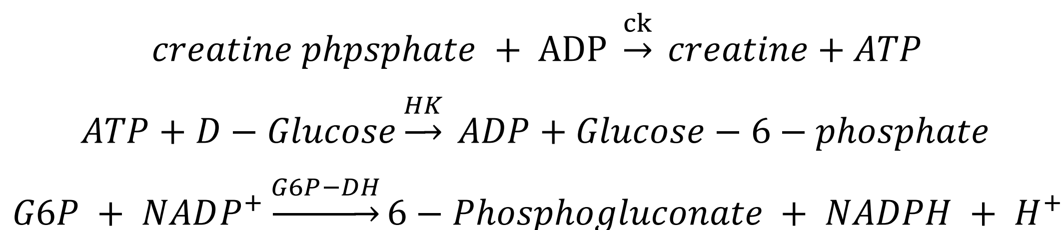
- **Principle**

Enzyme-Linked Immunosorbent Assay (ELISA) is used for determination of serum level of coenzyme Q10. The plate has been pre-coated with Human CoQ10 antibody. CoQ10 present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Human CoQ10 Antibody is added and binds to CoQ10 in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated CoQ10 antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and colour develops in proportion to the amount of Human CoQ10. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm (128).

2.4.2 Measurement of Serum creatine kinase

- **Principle**

Kinetic determination of the creatine kinase based upon IFCC and DGKC recommendations. Creatine kinase (CK) catalyses the reversible transfer of a phosphate group from phosphocreatine to ADP (135). This reaction is coupled to those catalyzed by hexokinase (HK) and glucose-6-phosphate dehydrogenase (G6P-DH):



$$creatine\ phpsphate + \text{ADP} \xrightarrow{\text{ck}} creatine + ATP$$

$$ATP + D - Glucose \xrightarrow{HK} ADP + Glucose - 6 - phosphate$$

$$G6P + NADP^{+} \xrightarrow{G6P-DH} 6 - Phosphogluconate + NADPH + H^{+}$$

The photometric rate of NADPH production is proportional to the catalytic concentration of CK in the sample (130).

- **Clinical Significance**

Creatine kinase is a cellular enzyme with wide tissue distribution in the body. Its physiological role is associated with adenosine triphosphate (ATP) generation for contractile or transport systems. Elevated CK values are observed in diseases of skeletal muscle and after myocardial infarction (131,132)

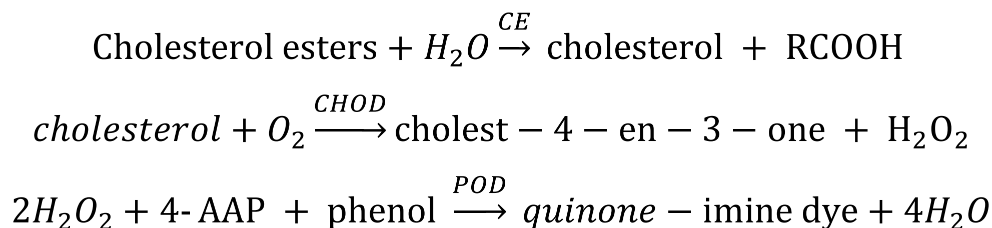
2.4.3 Measurement of Serum thyroid stimulating hormone (TSH)

Immunoassay for the in vitro quantitative determination of TSH in human serum was used in this study. The electrochemiluminescence immunoassay “ECLIA” was intended for the use on Elecsys and cobas e immunoassay analyzers and the principle was the same as described for prolactin hormone (133).

2.4.4 Determiination of lipid profile.

2.4.4.1 measurment of total cholesterol (TC)

In vitro quantitative enzymatic colorimetric method was used for the determination of total cholesterol in serum on cobas integra systems. Cholesterol esters are cleaved by the action of cholesterol esterase (CE) to yield free cholesterol and fatty acids. Cholesterol oxidase (CHOD) then catalyzes the oxidation ;-of cholesterol to cholest-4-en-3-one and hydrogen peroxide. In the presence of peroxidase (POD), the hydrogen peroxide formed effects the oxidative coupling of phenol and 4-aminoantipyrine (4- AAP) to form a red quinone-imine dye



$$\text{Cholesterol esters} + H_2O \xrightarrow{CE} \text{cholesterol} + \text{RCOOH}$$

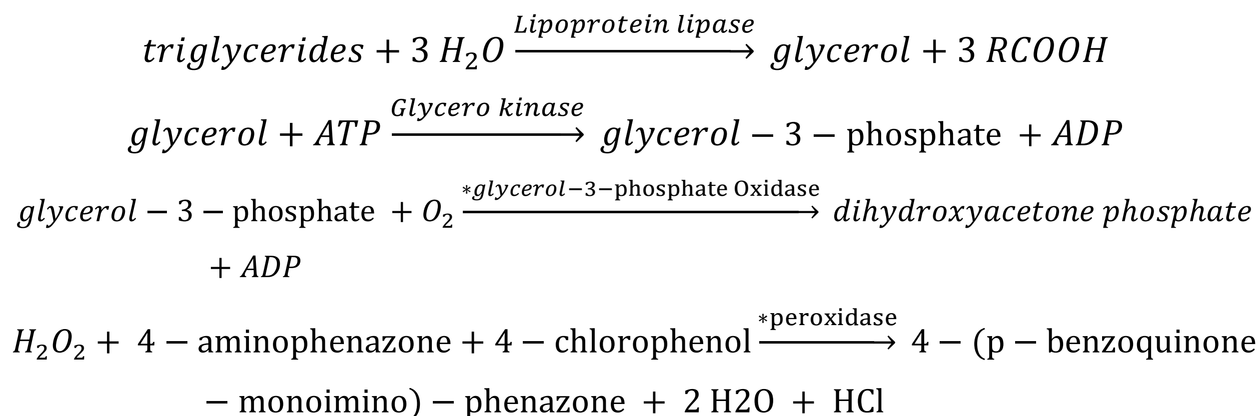
$$cholesterol + O_2 \xrightarrow{CHOD} \text{cholest} - 4 - \text{en} - 3 - \text{one} + \text{H}_2\text{O}_2$$

$$2H_2O_2 + \text{4-AAP} + \text{phenol} \xrightarrow{POD} quinone - \text{imine dye} + 4H_2O$$

The ratio of the cholesterol concentration to the dye's color intensity is straight proportional. The rise in absorbance at 512 nm was measured to make the determination (134).

2.4.4.2 measurement of Triglyceride (TG)

Triglyceride in serum was determined using an in vitro quantitative enzymatic colorimetric technique using Cobas integra systems (135).



$$triglycerides + 3\,H_2O \xrightarrow{Lipoprotein\ lipase} glycerol + 3\,RCOOH$$

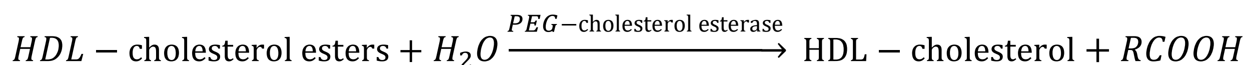
$$glycerol + ATP \xrightarrow{Glycero\ kinase} glycerol - 3 - \text{phosphate} + ADP$$

$$glycerol - 3 - \text{phosphate} + O_2 \xrightarrow{*glycerol-3-\text{phosphate Oxidase}} dihydroxyacetone\ phosphate + ADP$$

$$H_2O_2 + 4 - \text{aminophenazone} + 4 - \text{chlorophenol} \xrightarrow{*\text{peroxidase}} 4 - (\text{p} - \text{benzoquinone} - \text{monoimino}) - \text{phenazone} + 2\,\text{H2O} + \text{HCl}$$

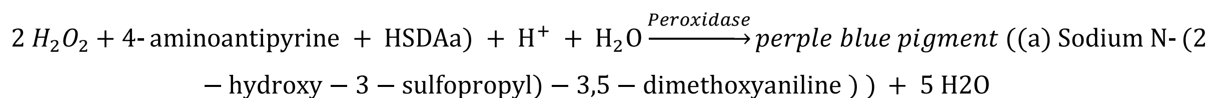
2.4.4.3 Assessment of Serum High Density Lipoprotein (HDL)

In vitro quantitative enzymatic colorimetric method was used for the determination of HDL in serum on cobas integra systems. In the presence of magnesium ions and dextran sulfate, water-soluble complexes with LDL, VLDL, and chylomicrons are formed which are resistant to PEG- modified enzymes. The cholesterol concentration of HDL-cholesterol was determined enzymatically by cholesterol esterase and cholesterol oxidase coupled with PEG to the amino groups (approximately 40 %). Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by cholesterol esterase. In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to Δ4-cholestenone and hydrogen peroxide (136).



$$HDL - \text{cholesterol esters} + H_2O \xrightarrow{PEG-\text{cholesterol esterase}} \text{HDL} - \text{cholesterol} + RCOOH$$

$$\text{HDL} - \text{cholesterol} + O_2 \xrightarrow{PEG-\text{cholesterol oxidase}} \Delta 4 - \text{cholestenone} + H_2O_2$$

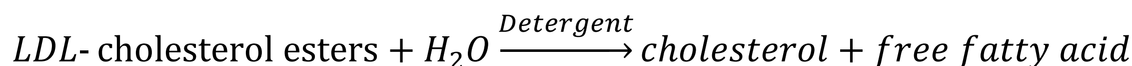


$$2\,H_2O_2 + \text{4-aminoantipyrine} + \text{HSDAa)} + \text{H}^+ + \text{H}_2\text{O} \xrightarrow{Peroxidase} perple\ blue\ pigment\ \text{((a) Sodium N-(2} - \text{hydroxy} - 3 - \text{sulfopropyl)} - 3{,}5 - \text{dimethoxyaniline))} + 5\ \text{H2O}$$

The amount of blue quinoneimine dye produced has a direct correlation with the level of HDL cholesterol in the blood. By monitoring the rise in absorbance at 583 nm, it was identified.

2.4.4.4 Assessment of Serum Low Density Lipoprotein (LDL)

Cholesterol esters and free cholesterol in LDL are measured based on a cholesterol enzymatic method using cholesterol esterase and cholesterol oxidase in the presence of surfactants which selectively solubilize the only LDL. The enzyme reactions to the lipoproteins other than LDL are inhibited by surfactants and a sugar compound. Cholesterol in HDL, VLDL, and chylomicron is not determined.



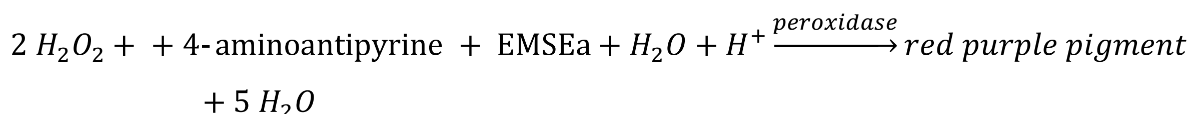
$$LDL\text{- cholesterol esters} + H_2O \xrightarrow{Detergent} cholesterol + free\ fatty\ acid$$

(Selective micellary solubilization)

By using cholesterol esterase, cholesterol esters are quantitatively converted into free cholesterol and fatty acids.

$$\text{LDL- cholesterol} + \text{O}_2 \xrightarrow{\text{cholesterol oxidase}} \Delta\text{4- cholestenone} + \text{H}_2\text{O}_2$$

Cholesterol oxidase converts cholesterol into 4-cholestenone and hydrogen peroxide when oxygen is present.



$$2\,H_2O_2 + + \text{4-aminoantipyrine} + \text{EMSEa} + H_2O + H^+ \xrightarrow{peroxidase} red\ purple\ pigment + 5\,H_2O$$

When peroxidase is present, the hydrogen peroxide produced combines with EMSE and 4-aminoantipyrine to produce a red-purple color. This dye's color intensity is evaluated using a photometer in direct proportion to the level of cholesterol in the blood (137).

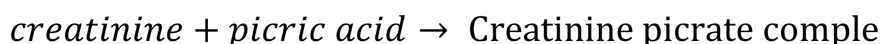
2.4.5 Determination of Renal function

2.4.5.1 Measurement of Creatinine

Creatinine is a waste product excreted by the kidneys mainly by glomerular filtration. The concentration of creatinine in plasma of a healthy individual is fairly constant, independent from water intake, exercise and rate of urine production. Therefore, increased plasma creatinine values always indicate decreased excretion, i.e. impaired kidney function. The creatinine clearance enables a quite good estimation of the glomerular filtration rate (GFR) which allows better detection of kidney diseases and monitoring of renal function. For this purpose creatinine is measured simultaneously in serum and urine collected over a defined time period (138).

- **Principle**

In an alkaline picrate solution, creatinine takes the form of an orange-red colored complex. The ratio of the difference in absorbance at specific conversion periods to the sample's creatinine content(139).



$$creatinine + picric\ acid \rightarrow \text{Creatinine picrate comple}$$

2.4.6 Determination of Body Mass Index

A person's weight and height are used to calculate their body mass index (BMI). The BMI was defined as the body weight divided by the square of the body height. It is generally expressed in kilograms per square meter (kg/m^2) because mass is measured in kilograms and height is measured in meters.

BMI=Weight (Kg)/ {Height (m)}^{2}

Normal weight of BMI value falls between 18.5-24.9 while overweight value between 25-30 and obese value above 30 (140).

2.4.7 Statistical analysis

The data of participants in this study were converted into a computerized database, revised for errors or inconsistencies, and then managed, processed, and analysed by using the statistical package for social sciences (SPSS) version 28, IBM, US.

Scale variables presented in mean, standard deviation (SD), while descriptive statistics for nominal (categorical) variables represented as frequency (number of participants) and proportion (percentage). Scales variables like Age and weight that follow the statistical normal distribution, so parametric test was applied. Student's test for two independent samples was used to compare means between groups. Analysis of variances (one way ANOVA) was used to compare more than two means. Chi square was used to measure the association between categorical variables. Fisher's exact test was used as an alternative when the chi square was inapplicable.

Finally, results and findings were presented in tables and or figures with an explanatory paragraph for each table or figure.

CHAPTER THREE

RESULTS

3.1. Demographic Characteristic of patients:

Demographic characteristics for patients participated in the study showed that the age categories reveals that the age group (48—65 years) are the higher percentage among other group. As shown in the table below, where males constituted 46% compared to females, in addition to that, BMI was the highest percentage among the group of overweighed people as shown in table (3-1).

Table (3-1): Descriptive of the Demographic and of the study population (n= 150).

Variable		N	%
Age (Years)	30 – 37 Years	16	10.7
	38 – 47 Years	35	23.3
	48 – 65 Years	99	66.0
Gender	Male	69	46
	Female	81	54
BMI Category	Normal weight	42	28.0
	Over weight	70	46.7
	Obese	38	25.3
Duration Treatment	1 – 24 Months	104	69.3
	25 – 48 Months	32	21.3
	49 – 72 Months	9	6.0
	73 – 96 Months	5	3.3
Smoking	Yes	41	27.3
	No	109	72.7
muscle cramps	Yes	47	31.3
	No	103	68.7
Weakness	Yes	60	40.0
	No	90	60.0
No Symptoms	Yes	49	32.7
Data Presented by numbers and percentage			

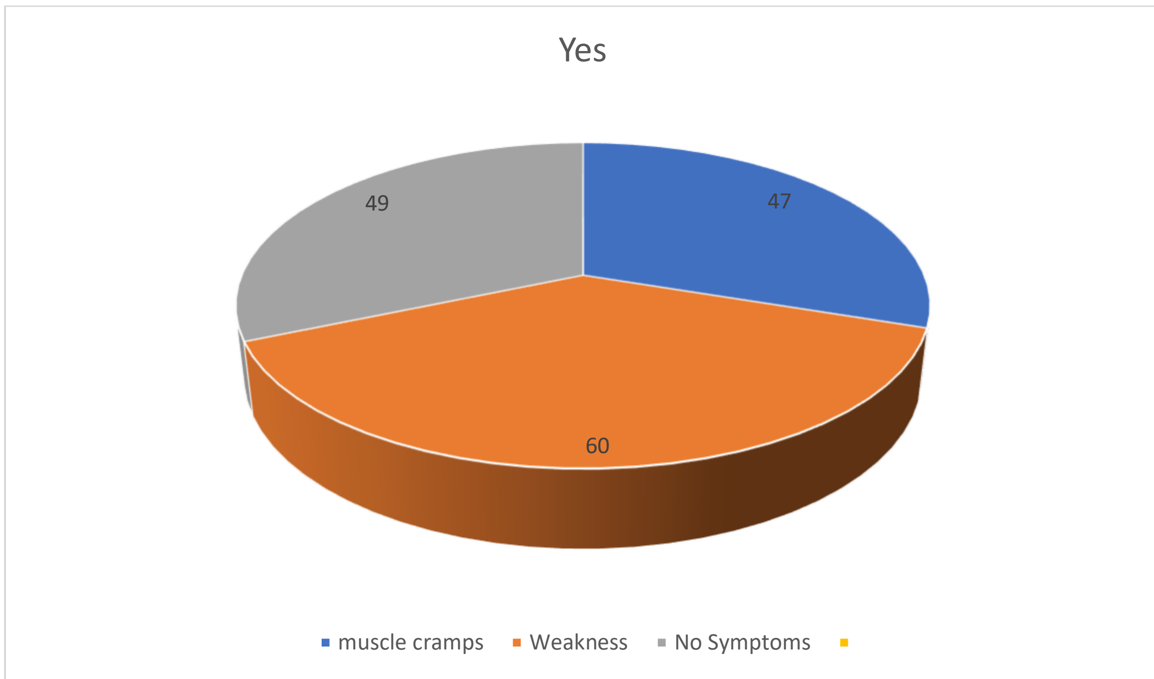


Figure (3-1): Frequencies of Symptoms in patients treated with atorvastatin.

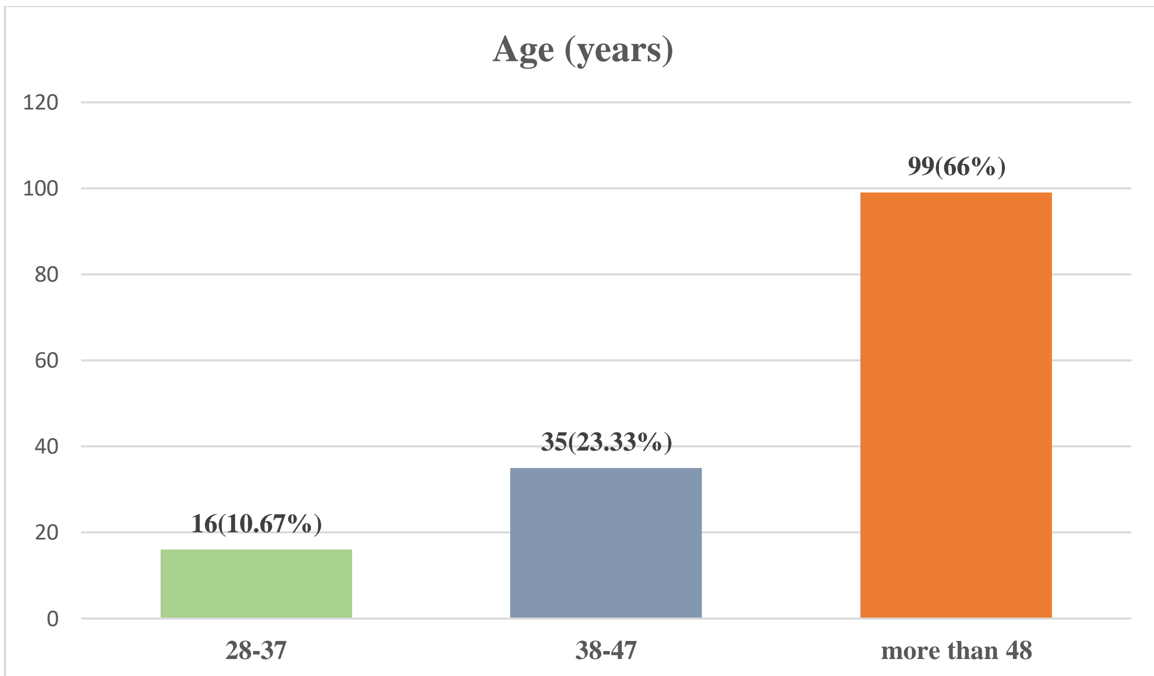


Figure (3-2): Frequencies of age groups of participants.

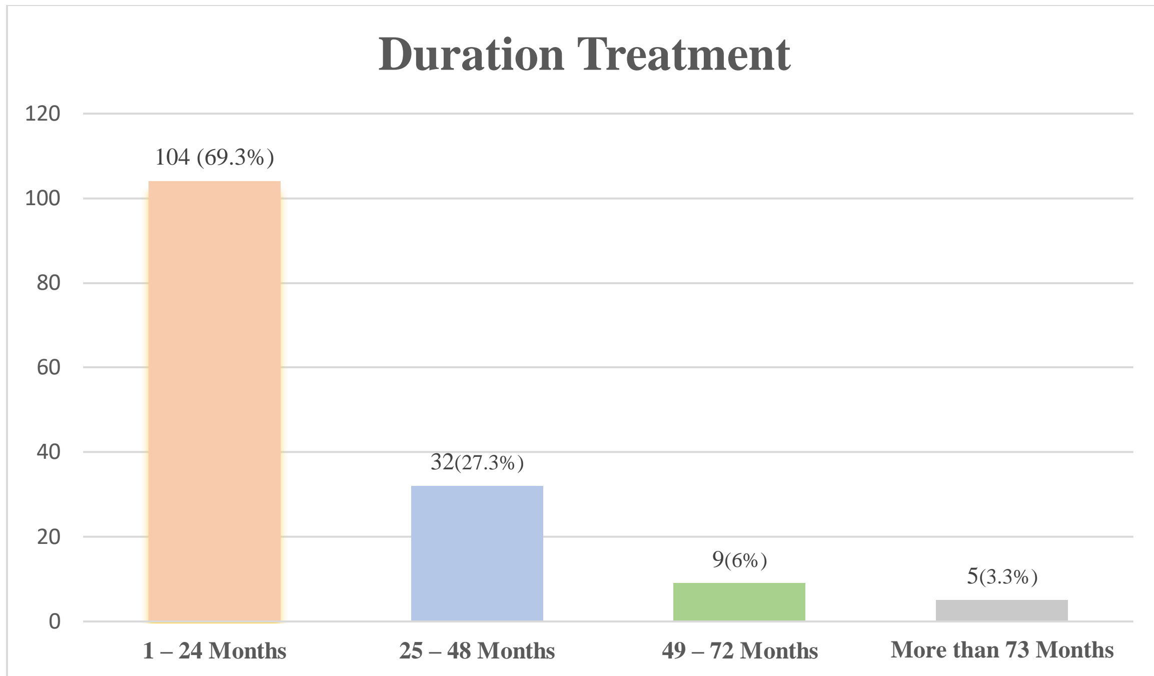


Figure (3-3): Frequencies of duration treatment of participants.

In the table(3-2) , the mean level of serum biomarkers was also examined based on the duration of statin treatment in hyperlipidaemia patients.

A statistically significant difference was found among mean of COQ10 duration of treatment groups ($p < 0.05$). Post hoc testing using LSD adjustment showed that the mean COQ10 for the (49 – 72 Months) group (10.44 ± 8.35) is significantly lower than that of other 3 groups, A statistically significant difference was found among mean of Cr duration of treatment groups ($p < 0.05$). Post hoc testing using LSD adjustment showed that the mean Cr for the (73-96 months) group (1.080 ± 0.144) is significantly higher than that of other 3 groups. A statistically significant difference was also found among mean of CK and duration of treatment groups ($p < 0.05$). Post hoc testing using LSD adjustment showed that the mean CK for the (73-96 months) group (171.80 ± 89.486) is significantly higher than that of other 3 groups. No significant difference was found between other mean of parameters and duration of treatment groups, ($p > 0.05$).

Table (3-2): Mean difference of biochemical parameters between duration of treatment groups.

biochemical parameters	Duration Treatment Group 2				P value
	1 – 24 Months	25 – 48 Months	49 – 72 Months	73 – 96 Months	
COQ10(ng/ml)	11.73 ± 5.61	11.03 ± 6.96	10.44 ± 8.35*	10.01 ± 11.33	**0.05 [S]**
Cr(mg/dL)	0.80 ± 0.38	0.81 ± 0.25	0.82 ± 0.21	1.08 ± 0.144*	**0.031[S]**
CK(U/L)	126.95± 52.44	152.66± 55.56	94.11 ± 27.53	171.80± 89.48*	**0.019[S]**
TSH(µlU/mL)	1.94 ± 1.16	1.70 ± 0.72	1.75 ± 0.85	1.43 ± 0.75	0.771[NS]
Chol(mg/dL)	164.52 ± 50.137	166.44 ± 56.402	156.00± 37.89	175.40 ± 28.51	0.534[NS]
TG(mg/dL)	154.44± 68.11	163.22± 76.28	170.33±62.48	223.40± 110.73	0.390[NS]
LDL(mg/dL)	90.67 ± 36.76	89.238 ± 42.965	77.00± 25.84	86.640± 28.07	0.741[NS]
HDL(mg/dL)	40.52 ±10.12	40.37 ± 14.45	45.56 ± 12.27	42.80 ± 8.40	0.440[NS]

Results are presented as mean ± SD, p<0.05 considered significantly different, [S]= Significant, [NS]= Non significant

3.2. Results of Amplification Reactions

3.2.1. Genotyping of COQ2 gene (rs6818847)

The results of gene polymorphism rs6818847 was a clear band with a molecular size 120 bps. Figure (3-4) The size of amplicon was determined by compare with DNA ladder 100 - 1000 bp.

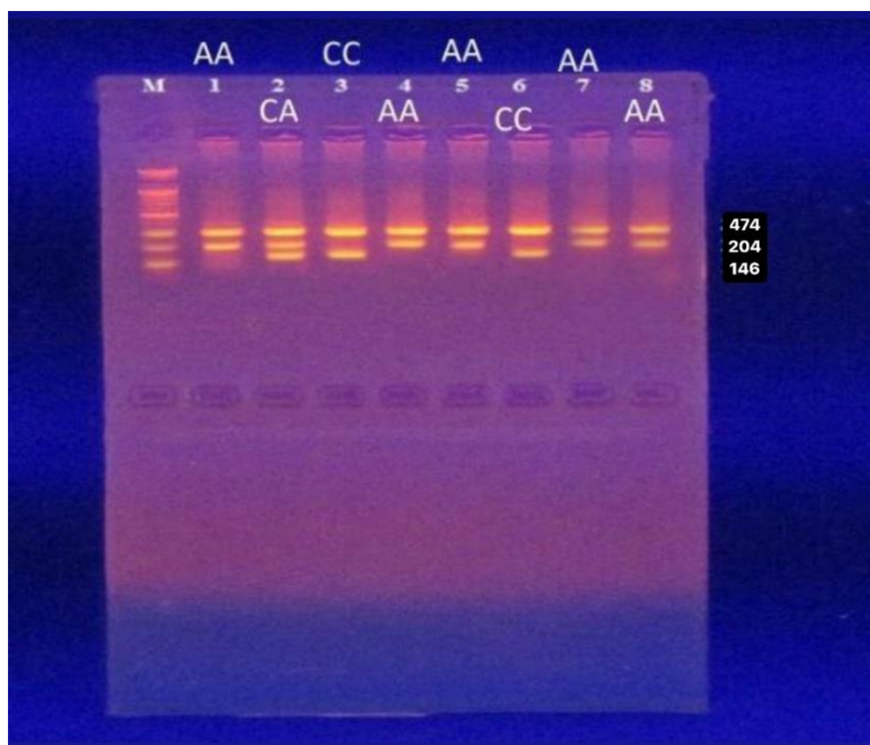


Figure (3-4): Genotyping of gene polymorphism rs6818847.

Genetic polymorphism of gene for rs6818847 SNP which were classified into three genotypes:

1. The major genotype group (CC) homozygous for the allele C.
2. The minor genotype group (AA) homozygous for the allele A.
3. Heterozygous (CA).

The distribution of genotyping groups of patients shows in table (3-3).

Table (3-3): Distribution of gene polymorphism rs6818847 SNP different genotype in patients.

Variable	Group	Frequency	Percentage
Genotype	**CC (wild)**	50	33.3%
	CA (hetero)	23	15.3%
	AA (mutant)	77	51.4%
Data Presented by numbers and percentage			

The result of comparison between observed and anticipated values for both SNP rs with rs6818847 SNP in the tested population were shown in figure (3-5). The distribution and percentage of individuals having rs6818847 SNP differ from those expected under Hardy–Weinberg equilibrium {number of observed vs expected were: {CC (50, 25.215); AA (77, 52.215); CA (23, 72.57) (goodness-of-fit $\chi 2$ for rs6818847 gene 69.987, $P < 0.001$} and therefore it was statistically significant table (3-4).

Table (3-4): Hardy–Weinberg equilibrium for rs 6818847 in patients.

Genotypes			Alleles		Hardy–Weinberg equilibrium X^2 test
			C	A	
Symbol	Frequency	%	0.41	0.59	69.987 **P < 0.001 [S]**
CC (Wild)	50	33.3			
CA (hetero)	23	15.3			
AA (Mutant)	77	51.4			
Total	150				

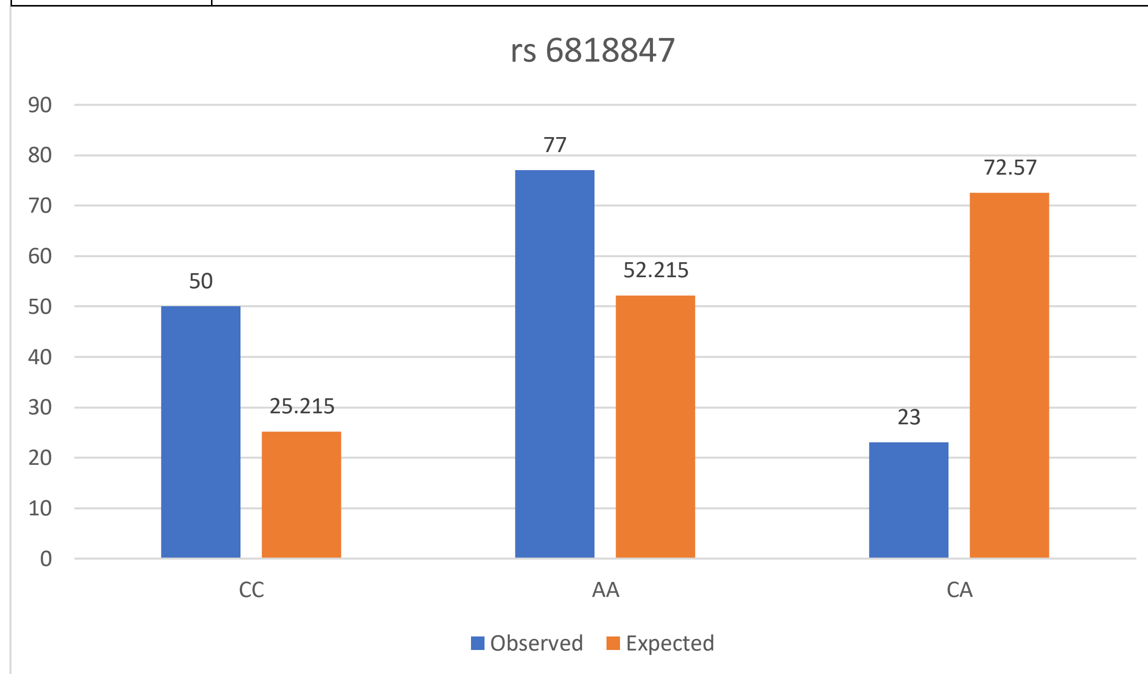


Figure (3-5): Observed (Obs.) vs expected (Exp.) genotype frequencies % of rs6818847 gene among individuals' sample.

3.2.2. Relationship between demographic characteristics and rs 6818847 single nucleotide polymorhisim.

To show the difference between demographic characteristics (mean) and rs 6818847 SNP (table (3-5)), by performing a one-way ANOVA test to compare the mean age, weight, height, BMI, duration of treatment. No statistically significant difference was found among mean of demographic characteristics (p > 0.05).

A chi-square test was conducted between gender, smoking, and symptoms between rs 6818847 SNP, there was no statistically significant difference between them (p > 0.05).

Table (3-5): Difference between demographic characteristic mean in rs 6818847 SNP.

Demographic parameters			**Patient Genotype (N=150)**			**P value**
			CC (N=50)	**CA (N=23)**	**AA (N=77)**	
Gender	**Male**		20(40%)	10(43.5%)	39(50.6%)	0.471 [NS]
	Female		30(60%)	13(56.5%)	38(49.4%)	
Symptom	**Muscle cramp**	**Yes**	18(36%)	4(17.4%)	25(32.5%)	0.268 [NS]
		No	32(64%)	19(82.6%)	52(67.5%)	
	weakness	**Yes**	15(30%)	10(43.5%)	35(45.5%)	0.207 [NS]
		No	35(70%)	13(56.5%)	42(54.5%)	
	Symptom	**Yes**	20(40%)	9(39.1%)	20(26%)	0.199 [NS]
		No	30(60%)	14(60.9%)	57(74%)	
Results are presented as mean ± SD, or n= number of subjects and percentage, p<0.05 considered significantly different, [S]= Significant, [NS]= Non significant						

3.2.3. Effect of treatment with Statins on Lab. parameters having rs 6818847 SNP.

To show the difference between mean of lab. Finding of (COQ10, CK, TSH, and Cr) between rs6818847 SNP groups (table (3-6)), by comparing the mean using a one-way ANOVA test. A statistically significant difference was found among mean of COQ10 between rs6818847 SNP groups ($p < 0.001$). Post hoc testing using LSD adjustment showed that the mean COQ10 for the CC allele (14.91±7.31) is significantly higher than that of other 2 alleles. No significant difference was found between other mean of parameters and rs6818847 SNP groups, ($p > 0.05$).

Table (3-6): difference between laboratory finding mean in rs6818847 SNP.

LAB parameters	*COQ2 gene* rs 6818847 SNP (N= 150)			P value
	CC (N=50)	CA (N=23)	AA (N=77)	
COQ10	14.91±7.31*	9.58±4.52	9.75±4.97	**<0.001 [S]**
CK	119.54±56.96	138.57±46.3	137.64±55.64	0.159[NS]
Cr	0.86±0.46	0.78±0.24	0.79±0.79	0.390 [NS]
TSH	1.80±1.10	1.57783±0.8	1.9816±1.05	0.245 [NS]
Results are presented as mean ± SD, p<0.05 considered significantly different, [S]= Significant, [NS]= Non significant,* = signifigant group				

3.2.4. Effect of treatment with Statins on lipid profile parameters having rs6818847 SNP:

To show the difference between mean of lipid profile Finding of (Cholesterol, triglyceride, LDL, and HDL) between rs6818847 SNP groups (table (3-7)), by comparing the mean using a one-way ANOVA test. No significant

difference was found between lipid profile finding mean in rs6818847 SNP groups, ($p > 0.05$).

Table (3-7): Effect of COQ2 gene polymorphism rs6818847 on lipid profile parameter.

Lipid profile parameters	*COQ2 gene* rs 6818847 SNP (N= 150)			P value
	CC (N=50)	CA (N=23)	AA (N=77)	
CHOL	170.08±51.54	155.91±42.6	163.99±51.326	0.525 [NS]
TG	161.90±79.67	156.52±55.4	158.96±71.063	0.952 [NS]
LDL	90.74±33.93	83.41±37.03	90.338±39.55	0.705 [NS]
HDL	41.64±11.26	41.26±9.9	40.25±11.62	0.782 [NS]
Results are presented as mean ± SD, p<0.05 considered significantly different, [S]= Significant, [NS]= Non significant				

3.3. Genotyping of COQ2 gene (rs6535454)

Genetic polymorphism of gene for rs6535454, which observed were classified into three genotypes:

1. The major genotype group (AA) homozygous for the allele A.
2. The minor genotype group (GG) homozygous for the allele G.
3. The heterozygous (AG).

Table (3-8) and figure (3-7) summarizes the distribution of genotyping groups of rs6535454 in patients.

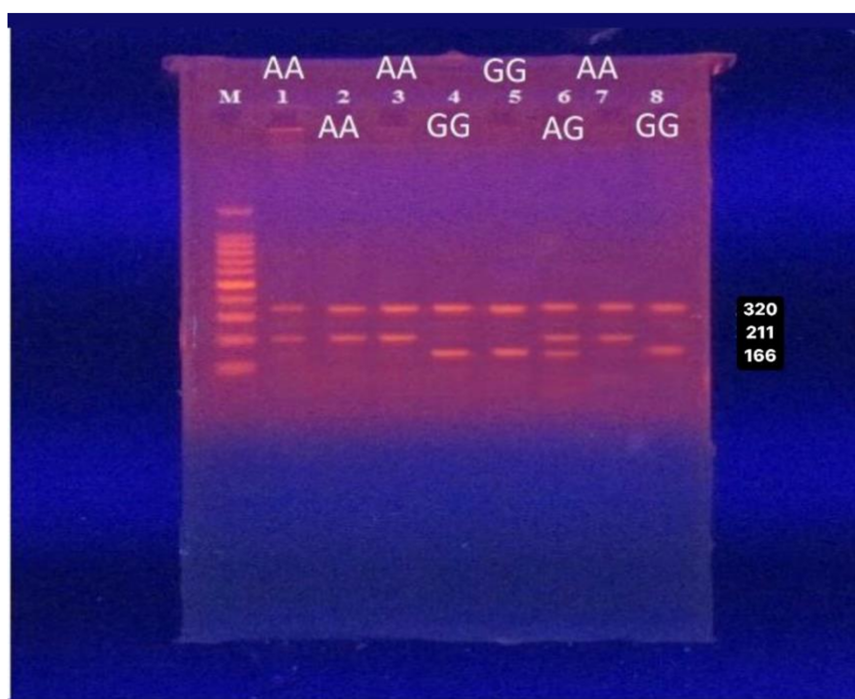


Figure (3-7): Genotyping of gene polymorphism rs 6535454.

Table (3-8): Distribution of gene polymorphism rs 6535454 different genotypes in Patients.

Variable	Group	Frequency	Percentage
Genotype	**AA (wild)**	63	42%
	AG (hetero)	37	24.7%
	GG (mutant)	50	33.3%
Data Presented by numbers and percentage			

In same way SNP rs6535454 differ from those expected under Hardy–Weinberg equilibrium (figure 3-8), number of observed vs expected were: {AA (63, 44.28); GG (50, 31.29); AG (37, 74.38) (goodness-of-fit $\chi 2$ for SNP rs6535454 gene = 22.234, $P < 0.001$} and therefore it was statistically significant table (3-9).

Table (3-9): Hardy–Weinberg equilibrium for rs 6535454 in patients.

Genotypes			Alleles		Hardy–Weinberg equilibrium X^2 test
			A	G	
Symbol	Frequency	%	0.5433	0.4567	37.940 P < 0.001 [S]
AA(Wild)	63	42			
AG (Hetero)	37	24.7			
GG (Mutant)	50	33.3			
Total	150				

Figure (3-8): Observed (Obs.) vs expected (Exp.) genotype frequencies % of rs 6535454 gene among individuals' sample.

3.3.1. Relationship between demographic characteristics and rs 6535454 SNP polymorphisim.

To show the difference between demographic characteristics (mean) and rs6535454 SNP (table 3-10), by performing a one-way ANOVA test to compare the mean age, weight, height, BMI, duration of treatment. A significant difference was found between mean weight of patient and rs 6535454 SNP, (p = 0.015), post hoc

testing using LSD adjustment showed that the mean weight for the AA SNP (79.1±13.48) is higher than that AG SNP (71.35±11.63).

No statistically significant difference was found among other mean of demographic characteristics ($p > 0.05$).

A chi-square test was conducted between gender, smoking, and symptoms between rs 6535454 SNP, there was no statistically significant difference between them ($p > 0.05$).

Table (3-10): difference between demographic characteristic mean in rs 6535454 SNP.

<table>
<tr><th colspan="3" rowspan="2">Demographic parameters</th><th colspan="3">Patient Genotype (N=150)</th><th rowspan="2">P value</th></tr>
<tr><th>AA
(N=63)</th><th>AG
(N=37)</th><th>GG
(N=50)</th></tr>
<tr><td rowspan="2">Gender</td><td colspan="2">Male</td><td>29(46%)</td><td>15(40.5%)</td><td>50(50%)</td><td rowspan="2">0.682 [NS]</td></tr>
<tr><td colspan="2">Female</td><td>34(54%)</td><td>22(59.5%)</td><td>25(50%)</td></tr>
<tr><td rowspan="6">Sympto m</td><td rowspan="2">Muscle cramp</td><td>Yes</td><td>22(34.9%)</td><td>9(24.3%)</td><td>16(32%)</td><td rowspan="2">0.54 [NS]</td></tr>
<tr><td>No</td><td>41(65.1%)</td><td>28(75.7%)</td><td>34(68%)</td></tr>
<tr><td rowspan="2">weakness</td><td>Yes</td><td>25(39.7%)</td><td>14(37.8%)</td><td>21(42%)</td><td rowspan="2">0.924 [NS]</td></tr>
<tr><td>No</td><td>38(60.3%)</td><td>23(62.2%)</td><td>29(58%)</td></tr>
<tr><td rowspan="2">None</td><td>Yes</td><td>22(34.9%)</td><td>14(37.8%)</td><td>13(26%)</td><td rowspan="2">0.448 [NS]</td></tr>
<tr><td>No</td><td>41(65.1%)</td><td>23(62.2%)</td><td>37(74%)</td></tr>
<tr><td colspan="7">Results are presented as mean ± SD, or n= number of subjects and percentage, p<0.05 considered significantly different, [S]= Significant, [NS]= Non significant</td></tr>
</table>

3.3.2. Effect of treatment with Statins on laboratory parameters having rs 6535454 SNP.

To show the difference between mean of lab. Finding of (COQ10, CK, TSH, and Cr) between rs6535454 SNP groups (table 3-11), by comparing the mean using a one-way ANOVA test. A statistically significant difference was found among mean of COQ10 between rs6535454 SNP groups ($p < 0.001$). Post hoc testing using LSD adjustment showed that the mean COQ10 for the AA allele (14.40±6.98) is significantly higher than that of other 2 alleles. No significant difference was found between other mean of parameters and rs6535454 SNP groups, ($p > 0.05$).

Table (3-11): difference between laboratory finding mean in rs 6535454 SNP.

LAB parameters	*COQ2 gene* rs **6535454 SNP (N= 150)**			P value
	AA (N=63)	AG (N=37)	GG (N=50)	
COQ10	14.40±6.98*	10.23±5.6	8.63±3.68	**<0.001[S]**
CK	119.35±49.91	141.86±56.1	139.88±58.66	0.062[NS]
Cr	0.85±0.401	0.82±0.35	0.76 ±0.23	0.398[NS]
TSH	1.79±1.07	1.72±1.02	2.03±1.05	0.335[NS]
Results are presented as mean ± SD, p<0.05 considered significantly different, [S]= Significant, [NS]= Non significant				

3.3.3. Effect of treatment with Statins on lipid profile parameters having rs 6535454.

To show the difference between mean of lipid profile Finding of (Cholesterol, triglyceride, LDL, and HDL) between rs 6535454 SNP groups (table 3-12), by comparing the mean using a one-way ANOVA test. A statistically significant difference was found among mean of HDL between rs 6535454 SNP groups ($p < 0.046$). Post hoc testing using LSD adjustment showed that the mean HDL for the GG allele (43.78±12.59) is significantly higher than that of AA allele (38.52±9.01). No significant difference was found between other mean of parameters and rs 6535454 SNP groups, ($p > 0.05$).

Table (3-12): difference between lipid profile mean in rs6535454 .

Lipid profile parameters	*COQ2 gene* rs **6535454 SNP (N= 150)**			P value
	AA (N=63)	**AG (N=37)**	**GG (N=50)**	
CHOL	165.65±50.36	155.27±47.4	170.72±51.56	0.360[NS]
TG	173.08±84.38	140.19±49.7	156.88±64.99	0.080[NS]
LDL	88.25±37.68	83.23±33.92	95.45±38.77	0.303[NS]
HDL	38.52±9.01*	40.93±11.9	43.78±12.59*	**0.046 [S]**
Results are presented as mean ± SD, p<0.05 considered significantly different, [S]= Significant, [NS]= Non significant				

3.4 Difference between both *COQ2 gene* rs 6818847 SNP & rs 6535454 SNP are mutant SNP and either rs 6818847 SNP or rs 6535454 SNP Mutant or no mutation:

To show the difference between both *COQ2 gene* rs 6818847 SNP & rs 6535454 SNP are mutant SNP and either rs 6818847 SNP or rs 6535454 SNP Mutant or no mutation, which were divided into two sub groups based on: Group 1 – Patients having Both rs 6818847 SNP & rs 6535454 mutant, Group 2- Patients who have Either rs 6818847 SNP or rs 6535454 Mutant or patients who not having neither rs 6818847 SNP nor rs 6535454 Mutant, in relation to demographic, lab. parameters, clinical parameters (mean) (table 3-14), by performing a student t-test. A statistically significant difference was found among mean of COQ10 (p= 0.001) , **HDL (P= 0.05)** and no statistically significant difference was found among other mean of parameters (p> 0.05).

Table (3-13): Representing the cross tabulation between rs6818847 SNP and rs6535454 SNP.

			SNP2			Total
			AA	**AG**	**GG**	
SNP1	**CC**	Count	29	14	7	50
		% of Toatal	19.3%	9.3%	4.7%	33.3%
	CA	Count	12	5	6	23
		% of Toatal	8.0%	3.3%	4.0%	15.3%
	AA	Count	22	18	37	77
		% of Toatal	14.7%	12.0%	24.7%	51.3%
Total		Count	63 42.0%	37 24.7	50 33.3%	150

SNP1*SNP2 Cross tabulation

Table (3-14): Difference between both rs 6818847 SNP & rs 6535454 mutant SNP and either rs 6818847 SNP or rs 6535454 Mutant or no mutation.

Parameters	Patient Genotype (N=150)		P value
	Either rs 6818847 SNP or rs 6535454 Mutant or no mutation (N=113)	Both rs 6818847 SNP & rs 6535454 mutant (N=37)	
COQ10	12.49±6.57	8.26±3.84	**0.001 [S]**
Cr	0.83±0.37	0.79±0.25	0.532 [NS]
CK	128.45±55.33	141.81±54.03	0.202 [NS]
TSH	1.77±1.03	2.14±1.09	0.061 [NS]
Chol	161.06±48.72	176.14±53.08	0.112 [NS]
TG	158.81±72.82	161.89±68.49	0.821 [NS]
LDL	86.81±35.26	97.34±42.21	0.136 [NS]
HDL	39.87±10.86	43.92±11.89	**0.05 [S]**
Results are presented as mean ± SD, p<0.05 considered significantly different, [S]= Significant, [NS]= non-significant			

3.5. Estimation of risk

3.5.1. Estimation of risk in COQ2 *gene* rs 6818847 SNP in regarding to the lab. parameters:

The odds ratios of the detected genotypes of COQ2 *gene* rs 6818847 SNP in the levels of COQ10, Cr, CK, TSH, Chol, TG, LDL, and HDL in the myopathic patients treated with statin Table (3-15).

The logistic analysis of the COQ2 *gene* rs 6818847 SNP of the myopathic patients concluded that the response to treatment (Statin) regarding COQ10 level was significantly related to the CC allele in comparison with AA allele (OR = 1.237, $p < 0.001$). No other significant effect on other parameters $p > 0.05$.

Table (3-15): The odds ratios of COQ2 *gene* rs 6818847 SNP with levels of lab parameters**.**

Variables	SNP	OR (95% CI)	p value
COQ10	**CC**	1.144 (1.061-1.233)	**< 0.001 [S]**
	CA	0.985 (0.878-1.104)	0.794 [NS]
	AA	1[a]	-
Cr	**CC**	1.491 (0.493-4.511)	0.479 [NS]
	CA	0.947 (0.187-4.791)	0.947 [NS]
	AA	1[a]	-
CK	**CC**	0.998 (0.991-1.006)	0.655 [NS]
	CA	0.999 (0.99-1.008)	0.802 [NS]
	AA	1[a]	-
TSH	**CC**	0.866 (0.598-1.253)	0.444 [NS]
	CA	0.64 (0.369-1.11)	0.112 [NS]
	AA	1[a]	-
Chol	**CC**	1.009 (0.992-1.026)	0.311 [NS]
	CA	0.995 (0.968-1.023)	0.724 [NS]
	AA	1[a]	-
TG	**CC**	0.999 (0.993-1.005)	0.796 [NS]
	CA	1.001 (0.993-1.01)	0.768 [NS]
	AA	1[a]	-
LDL	**CC**	0.99 (0.97-1.01)	0.339 [NS]
	CA	0.999 (0.968-1.03)	0.934 [NS]
	AA	1[a]	-
HDL	**CC**	1.005 (0.969-1.042)	0.794 [NS]
	CA	1.018 (0.968-1.07)	0.496 [NS]
	AA	1[a]	-
Results are presented [S]; Significant, [NS]; Non significant, OR: Odds Ratio, CI; Confidence Interval, 1[a]; reference category			

3.5.2.Estimation of risk in COQ2 *gene* rs 6535454 SNP in regarding to the lab. parameters:

The odds ratios of the detected genotypes of COQ2 *gene* rs 6535454 SNP in the levels of COQ10, Cr, CK, TSH, Chol, TG, LDL, and HDL in the myopathic patients treated with statin Table (3-16).

The logistic analysis of the COQ2 *gene* rs 6535454 SNP of the myopathic patients concluded that the response to treatment (Statin) regarding COQ10 and HDL level was significantly related to the AA allele in comparison with GG allele (OR = 1.144, $p < 0.001$) and (OR = 0.951, $p = 0.019$) respectively. No other significant effect on other parameters $p > 0.05$.

Table (3-16): The odds ratios of COQ2 *gene* rs 6535454 SNP with levels of lab.parameters.

Variables	SNP	OR (95% CI)	p value
COQ10	**AA**	1.237 (1.11-1.38)	**< 0.001 [S]**
	AG	1.112 (0.99-1.249)	0.074 [NS]
	GG	1[a]	-
Cr	**AA**	1.596 (0.422-6.035)	0.491 [NS]
	AG	1.666 (0.397-6.992)	0.485 [NS]
	GG	1[a]	-
CK	**AA**	0.997 (0.989-1.005)	0.518 [NS]
	AG	1.001 (0.993-1.01)	0.723 [NS]
	GG	1[a]	-
TSH	**AA**	0.812 (0.555-1.188)	0.284 [NS]
	AG	0.757 (0.493-1.164)	0.205 [NS]
	GG	1[a]	-
Chol	**AA**	1.001 (0.99-1.032)	0.313 [NS]
	AG	1.008 (0.985-1.032)	0.504 [NS]
	GG	1[a]	-
TG	**AA**	1.001 (0.994-1.008)	0.799 [NS]
	AG	0.995 (0.987-1.003)	0.213 [NS]
	GG	1[a]	-
LDL	**AA**	0.985 (0.962-1.009)	0.216 [NS]
	AG	0.985 (0.959-1.012)	0.278 [NS]
	GG	1[a]	-
HDL	**AA**	0.951 (0.912-0.992)	**0.019 [S]**
	AG	0.974 (0.932-1.017)	0.233 [NS]
	GG	1[a]	-
Results are presented [S]; Significant, [NS]; Non significant, OR: Odds Ratio, CI; Confidence Interval, 1[a]; reference category			

CHAPTER FOUR

DISCUSSION

4. Discussion

Myopathy is recognized as the most common adverse effect associated with statin therapy, ranging from myalgia (muscle symptoms without creatinine_kinase elevation) to myositis (muscle symptoms with increased creatinine kinase level). In rare situations, it can worsen to life threating rhabdomyolysis (muscle symptoms with marked elevation of creatinine kinase and myoglobinuria). The incidence of statins induced myopathy reported in previous studies ranged from 5% to 20% dependent on the criteria of diagnosis of myopathy and the type of study (141).

Few drugs are as widely used as the statins, which effectively decrease blood levels of cholesterol and protect against various cardiovascular diseases related to atherogenesis (142). Similarly, few drugs have generated as much controversy as the statins adverse effects, predominantly affecting skeletal muscle have been widespread and severe enough to force one pharmaceutical company to withdraw cerivastatin from the market (143). However, statins are still widely used and their safety is still debated. The common mechanism of action of these drugs, inhibition of cholesterol metabolism at the level of mevalonic acid, has the unintended consequence of impairing the synthesis of other compounds that share mevalonate as a precursor, such as dolichols and CoQ10 (ubiquinone) (144). Atorvastatin caused a rapid and substantial decrease of plasma CoQ10 concentrations, which was evident 14 days after the initiation of therapy and was even more marked after 30 days of therapy (145).

To the best of knowledge, this is the first study that investigated Iraqi atorvastatin taking patients for interindividual variability of COQ2 gene involved in statin induce myopathy.

4.1 Demographic data

Demographic characteristics for patients participated in the study were at atorvastatin 40 mg showed that, there age were within the range of (30-65 years), and the common age group was between (48-65 years) about 60% of patients.

Ageing is accompanied by major changes in body composition that can negatively affect functional status in older adults, including a progressive decrease in muscle mass, strength, and quality, accompanied by an increase in fat mass. Changes in skeletal muscle are especially important because muscle is essential for locomotion (146) .

So, the older age more than 50 years were associated with statistically significant increased risk of developing statin associated adverse muscle reactions (147). This study disagree with previous study, where there is no significant association between age groups and statin related muscle symptoms. While it was agree with ,where they observed no age dependent differences and it seems to unlikely that the oldest patients are at higher risk because the incidence of muscular symptoms in this group(98).

The gender distribution was about 46% for male and 54% female, the result show that, 40.3% of 69 male and 80.3% of 81 female patients have muscular symptoms, these reveal that the female gender are more likely to occurrence of statin associated muscle problems .Males in general are physically stronger when compared to females which could be due to more number of muscle mass and less body fat when compared to females. This attributed to many factors and the most important one is hormonal. Testosterone helps build lean body mass or muscle in males and also it increase muscle strength (148). These disagree with (149), which found that in regard to gender myopathy was more common in men than in women (31.4 percent vs. 22.6 percent), although it was in agreement with *skilliving et*

al.(2016)(145) Which showed that female had a higher frequency than male (17 percent vs. 12 percent).

Obesity triggers a cascade of events including increased adipocyte size and number of macrophages, more pro-inflammatory senescent cells in adipose tissue, increased inflammatory markers, reactive oxygen species, insulin resistance, and leptin along with lowered adiponectin, and, ultimately, loss of muscle mass and strength disproportionate to relatively greater body size. The skeletal muscle, is insufficient to transport the obese individual and leads to the development of muscle problems (150).

Most of participant in study were over weighted (46%). There is a positive correlation between body mass index and adverse muscle reaction that caused by atorvastatin use. Several researchers reported obesity as risk factor (151). Whereas others discovered that a statin-induced myopathy is more likely when a person is fragile and has a small body size (152). This study support the first observation, and it's also disagree with (152), where the lower BMI was statistically significant association with increase risk of developing muscle symptoms.

Mostly of the patients (about 69.3 %) were having a duration of treatment in range of (1-24) months. About 68.7% of the patients were reported to not having any muscle cramps while 60% were not having any Weakness. In statin-induced myopathy, the muscle pathology is non-specific, including fiber necrosis, degeneration, regeneration, and phagocytic infiltration. In certain instances, ragged red fibers, subsarcolemmal accumulations, lipid-filled vacuoles, and cyclo-oxygenase negative fibers are visible (153). Breakdown of the T-tubular membranes and subsarcolemmal fissuring are two examples of skeletal myocyte injury (separating the myo- filaments from the plasma membrane, but leaving the plasma membrane intact). These modifications take place even in patients who do not exhibit symptoms (154).

On the other hand, research was done on the influence of pharmacogenetics on patients from South India who developed statin-induced myopathy. Statin therapy is constrained by skeletal muscle toxicity brought on by increased systemic drug exposure. Up to 10% of statin-treated people may occasionally experience muscle pain or weakness (155).

Nearly 72% of the people under study were non-smokers, whereas 21% were active smokers. Smokers are less likely to engage in physical activity, which is secondary to the finding that they are less likely to experience muscle pain (156).

4.2 Biochemical investigation

For one hundred fifty patient the serum Coenzyme Q10, lipid profile (cholesterol, triglyceride, low density lipoprotein, and high density lipoprotein), renal function test (creatinine), creatinine kinase, and thyroid stimulating hormone were measured.

Coenzyme Q10 is a fat-soluble compound that is synthesized by the body and can be obtained from the diet. Coenzyme Q10 has a major function as an antioxidant and is an essential cofactor in the mitochondrial respiratory chain. The measurement is useful for the diagnosis of mitochondrial disorders and is used to monitor degenerative disorders such as Alzheimer and Parkinson disease as well as myopathy that caused by receiving statin therapy (157).

Statins reduce CoQ10 levels in two ways the first one is directly by inhibiting the HMG-CoA reductase, enzyme of the mevalonate pathway and the other one is reducing CoQ10 transport capacity due to decrease of LDL level (158).

In this study CoQ10 serum level was measured for all participants, the results was shown in table (3-2), that the CoQ10 level was markedly decreased as the duration of treatment increased in Iraqi population taking atorvastatin 40mg therapy however these change in CoQ10 level is statically significantwith duration of treatment. It is, therefore, not surprising that, starting with *Folkers et al*. of (1985)

(159). The impact of statins on the blood levels of CoQ10 in healthy individuals as well as hypercholesterolemic patients has been researched by numerous groups. Because different studies utilized various statins, dosages, and long- or short-term exposures, it can be challenging to compare the findings. Additionally, some studies used a small sample size, sometimes even just one person, while others used a larger series. Blood CoQ10 levels decreased by 50% and 54%, respectively, in a double-blind placebo-controlled trial (113) of healthy volunteers treated for 1 month with either pravastatin, 20 mg/dl (n = 10), or simvastatin, 20 mg/d (n = 10), for 4 weeks, while those receiving placebo showed no change.

In another large study,(160) A double-blind research including 45 hypercholesterolemic patients showed that there was a gradual decline in blood CoQ10 levels in all patients: after 18 weeks, the levels were 80% of baseline with pravastatin and 7% of baseline with lovastatin.

These finding was agree with *Tatjana et al.* of 2004 (161), where discovered that atorvastatin caused a rapid and significant decrease in plasma CoQ10 levels. This effect became apparent 14 days after the start of treatment and was even more pronounced 30 days later. Although it disagreed with *Bleske et al.* 2001 (162), The only study with negative results involved 12 healthy subjects show no change in blood CoQ10 level was found at the end of treatment.

Creatine kinase (CK), is the enzyme that catalyzes the reaction of creatine and adenosine triphosphate (ATP) to phosphocreatine and adenosine diphosphate (ADP). The phosphocreatine created from this reaction is used to supply tissues and cells that require substantial amounts of ATP, like the brain, skeletal muscles, and the heart, with their required ATP .Many conditions can cause imbalance in CK levels, including rhabdomyolysis, heart disease, kidney disease, or even certain medications (163).

Creatine kinase normally exists in the brain, skeletal muscles, heart tissue, and other organs. However, muscle damage and CK leakage into the bloodstream happen after statin use. Therefore, CK is a sign of muscle injury (164).

Serum CK level was checked in the present study the results that shown in table (3-2) had revealed that, CK level was increased as duration of statin treatment increased.

There is a statistically significant differences was found among mean of CK and duration of treatment groups (p=0.019). Post hoc testing using LSD adjustment showed that the mean CK for the (73-96 months) group (171.80 ± 89.486) is significantly higher than that of other three groups.

There was about one third of patients had on elevated CK level. In actuality, only seven of our patients showed moderately increased levels of CK, and all of them experienced muscle complaints and weakness upon examination. This result agree with (165) , where patients on atorvastatin shows CK level increased from (132.3 ± 120.9) (mean ± SD) at baseline to (159.7 ± 170.4) and (153.1 ± 139.4) at 3 and 6 months, respectively (P≤0.002 for both). More subjects on atorvastatin compared to placebo doubled their CK level at 6 months (P = 0.02).

This is in disagree with previous reports show normal serum CK levels were commonly found in patients with statin therapy (166-168).

Serum TSH level was evaluated for each participant before start the study and we exclude patients with lower TSH level, this indicated that these patients have hypothyroidism which is risk factor for statins induce myopathy and even spontaneous myopathy (169). Because hypothyroidism can produce generalized muscular enlargement, along with stiffness, weakness, and severe muscle cramps, serum CK levels are typically high (170).

The major observation of this study is that atorvastatin was influences the lipid parameters. Study participants who were evaluated for atorvastatin-induced

myopathy Based on the duration of treatment groups, were shown that lipid profile levels were not indicated any significant difference, although there was increasing in the levels of cholesterol, TG and decreasing in the levels of LDL with increasing the duration of treatment. Results were agreed with *Vondrakova., et al* of (2010) (171) who reported that an acute effect of intensive atorvastatin therapy on HDL-C and TG is opposite to long-term treatment: they have observed an acute decrease in HDL-C and an acute increase in TG levels. Current evidence for the acute effect of statins on lipid levels in such patients is poor. It concluded that intensive atorvastatin therapy initiated at admission of patients with ACS has a prompt acute effect on the lipid profile and that this effect differs from the long-term statin treatment.

Furtheremore, reduction in LDL-C levels was seen as the dose of the statin increased. The results of the study are in line with the previous studies (172,173).

4.3 Molecular analysis

The gene COQ2, encoding 4-hydroxybenzoate-polyprenyltransferase (coenzyme Q2), belongs to the candidates potentially influencing statin treatment tolerability. This enzyme is involved in the biosynthesis of coenzyme Q10 (CoQ10), in which depletion induced by statin treatment is implicated in the development of statin-associated muscle symptoms. Thus, polymorphisms in the COQ2 gene might explain susceptibility to statin associated muscle symptoms (174).

4.3.1 Examination the Hardy–Weinberg equilibrium for COQ2 rs 6818847 in patients treated with atorvastatin

Table (3-4) displayed the genotype and allele frequencies for rs 6818847. For both the recessive and the dominant models, the allele and genotype frequency distributions were in agreement with Hardy-Weinberg equilibrium ($p < 0.05$). A significant difference was discovered for these polymorphisms of the study participants. Based on the presence or absence of statin-induced myopathy, genetic information for rs 6818847 polymorphisms was divided into two groups. In the

study, homozygous wild genotypes of the rs 6818847 polymorphism had a genotype frequency of 33.3 percent, heterozygous people had a frequency of 15.3 percent, and homozygous mutants had a genotype frequency of about 51.4 percent. No explanation was provided. Additional research is needed to examine the other genotypes and to compare the plasma medication concentrations in patients receiving statin therapy.

4.3.2 Impact of COQ2 gene (C>A) (rs 6818847) in serum Creatine kinase level in patient taking atorvastatin

Based on creatine kinase level in table (3-6) the homozygous wild (CC) mean (119.54±56.96) and homozygous mutant genotype (AA) mean (137.64±55.64) and heterozygous genotype (CA) mean (138.57±46.3), the result show that in patients carry (AA) and (CA) genotypes there was elevation in creatine kinase level in compare with (CC) genotype in atorvastatin taking individuals.

Although this value did not rise to the extent that qualifies to be statistically significant, however, patients who have these genotypes are more sensitive to statin therapy, because the statin itself increase creatine kinase level by causing muscle damage and release this enzyme so, it exacerbated the condition.

In depend on the results we found that patients who taking statin therapy and have mutation in both SNP1 and SNP2 as shown in table (3-6) are more susceptible to appear of statin associated muscle problems , because in those patient the statin cause markedly decreased in coenzyme Q10 serum level, (who is very important enzyme for energy and muscle function) and in the same time in those patients statin caused damage to muscle and caused it to realise the creatine kinase enzyme as response to this damage, so they have markedly increase in this enzyme .

Because of the crucial function that CoQ10 plays in energy production through the mitochondrial respiratory chain and because of its anti-oxidant

capabilities, impaired CoQ10 synthesis may easily be the cause of the wide range of negative consequences that have been observed (175).

4.3. Impact of COQ2 gene (C>A) (rs6818847) in serum CoQ10 level in patient taking atorvastatin.

As shown in table (3-6) the effect of genetic polymorphism of COQ2 gene with (rs6818847) SNP group, the result establish that there is statistically significant association between CoQ10 serum level and homozygous wild genotype (CC) where higher level found in it (14.91±7.31). This indicate that the subjects that carry (CC) genotype not effect by statin and CoQ10 serum level stay high and within the normal range.

Whereas patients that have homozygous mutant genotype (AA) and heterozygous patient (CA) are more susceptible to statin related muscle symptoms considering to the decrease in COQ10 serum level (9.75±4.97) and (9.58±4.52) respectively.

In the study we found that the patient that have genetic polymorphism in that gene on this SNP was about 77 patients (51%) and those patients have (effect on symptoms) and also have negative effect on Coenzyme Q10 serum level.

The patients that carry the heterozygous (CA) of SNP (rs6818847) (23 patients) also have negative effect on serum CoQ10 level that cause depletion in energy and direct effect on muscle and its major cause of statin induce myopathy.

4.3.4 Examination the Hardy–Weinberg equilibrium for COQ2 gene (rs 6535454) in patients treated with atorvastatin.

Table (3-9) displayed the allele and genotype frequency distribution was in accordance with Hardy–Weinberg equilibrium ($p < 0.05$) for both recessive and dominant models. There was a significant difference found for these polymorphisms of the study subjects. Genetic data of rs6535454 polymorphisms were segregated

based on the presence and absence of statin-induced myopathy. The genotype frequency of the rs6535454 polymorphism was 42% for homozygous wild genotype, 24.7% for heterozygous individuals, and about 33.3% for homozygous mutants were observed in the study, no explanation were reported. Further studies are required to study the other genotypes and also to correlate the plasma concentrations of the drug in patients who are on statin therapy (176).

4.3.5 Impact of COQ2 gene (A>G) (rs6535454) in serum CoQ10 level in patient taking atorvastatin

Coenzyme Q10 enzyme is an important cofactor in mitochondrial respiration. Primary CoQ10 deficiency is clinically and genetically heterogenous condition, consider autosomal recessive and has been associated with myopathy (177).

In-patient on atorvastatin, reduction CoQ10 level is routinely observed (178) and a little decline in muscle CoQ10 has been recommended in some (179), but not other study (180). COQ2 encodes parahydroxy benzoate-polyprenyl transferase. Primary CoQ10 insufficiency and COQ2 have been linked (181).

COQ2 variants and in particular (rs6535454) (A>G) have been investigated, and have reported as shown in table (3-11) an association with statin induce myopathy by causing highly reduction in serum CoQ10 level particular in patients with homozygous mutant genotype (GG) which present in 50 patients with mean (8.63±3.68).

While in heterozygous genotype patients (AG) the COQ10 serum level mean was (10.23±5.6), it also decrease but less than (GG) patients. While the patients, which have wild type (AA) with mean (14.40±6.98) that have the ability to remain the CoQ10 level high and within the normal range. From the result the deduction

was that the COQ2 gene polymorphism in (rs6535454) can increase the risk of atorvastatin induce myopathy.

The study was agree with (182), reported significant association between statin intolerance and SNP (rs6535454) (p=0.047). While disagree with *Jaroslav et al.* (2017), where there is no correlation between the chance of developing statin-related muscle issues and the common polymorphism (rs6535454) within the COQ2 gene(183).

4.3.6 Impact of COQ2 gene (A>G) (rs6535454) in serum creatine kinase level in patient taking atorvastatin

Based on creatine kinase level in table (3-11) the homozygous wild (AA) mean (119.35±49.91) and homozygous mutant genotype (GG) mean (139.88±58.66) and heterozygous genotype (AG) mean (141.86±56.1), the result show that in patients carry (GG) and (AG) genotypes there was elevation in creatine kinase level in compare with (AA) genotype in atorvastatin taking individuals.

Although this value did not rise to the extent that qualifies to be statistically significant, however, patients who have these genotypes are more sensitive to statin therapy, because the statin itself increase creatine kinase level by causing muscle damage and release this enzyme so, it exacerbated the condition.

4.3.7 Effect of treatment with Statins on lipid profile parameters having rs 6818847 SNP& rs 6535454 SNP:

The association of mean levels of lipid profile (Cholesterol, triglyceride, LDL, and HDL) and rs 6818847 SNP groups was shown in table (3-7), the comparsion of the mean was performed using a one-way ANOVA test. No significant difference was found between lipid profile finding mean in rs 6818847 SNP groups, ($p > 0.05$). on the other hand, rs 6535454 SNP was illustrated asignificant difference with HDL levels as shown in table (3-12). Genome-wide association (GWA) studies provide a more comprehensive approach for identifying genetic loci associated with statin

response. GWA studies have identified many loci associated with plasma lipid and lipoprotein traits, including several not previously known to be related to lipoprotein metabolism (184-185). However, there is to date only one report of GWA of lipid response to statin treatment. This involved ~2,000 participants in the Treating with atorvastatin for eight weeks. However, no SNPs were identified from this analysis that were convincingly associated with atorvastatin-mediated lipid changes (P> 0.05) (186).

4.3.8 Difference between both *COQ2 gene* rs 6818847 SNP & rs 6535454 SNP are mutant SNP and either rs 6818847 SNP or rs6535454 SNP Mutant or no mutation:

When comparing the levels of markers in patients who have either mutant or no mutation in the rs 6818847 SNP or rs 6535454 (N=113) with patients who have mutation in both SNPs (rs 6818847 SNP & rs 6535454) (N=37), results were indicated that only COQ10 & HDL were differed significantly (p value=0.001)as shown in table (3-14).

Numerous gene loci are involved in the pharmacogenetics of statin treatment effectiveness and the prediction of problems; nevertheless, confirmatory investigations are typically lacking or uncommon (187-188). The most frequent side effect of statins was myopathy, and it is well recognized that this condition has a significant genetic component (189). Clear candidates for COQ gene variations that may have an impact on disease development exist. Mitochondrial para-hydroxybenzoate-polyprenyltransferase, an enzyme that the COQ2 gene encodes, catalyzes one step in the production of CoQ10 (ubiquinone). Statins have been reported to lower plasma amounts of CoQ10 (190).

CoQ10 deficiency is directly correlated with COQ2 gene locus polymorphisms (191), It appears to be crucial in a proposed pathway for statin-induced myopathy (192).

Surprisingly, only a few studies have addressed the role of the COQ2 gene variants as predictors of statin- induced muscle problems The first study on this topic (193) has discovered that the minor allele homozygotes of the rs6535454 polymorphisms have a significantly higher prevalence of statin intolerance. This finding was true for this SNPs and haplotypes. Even the genome-wide association studies (GWASS), which were focused on the detection of gene variations related with Myopathy, failed to identify the COQ gene as a prominent contender despite the possibility of convincing biological linkages between the COQ and Myopathy (194).

Also, there is an increasing number of observations doubting the harmlessness of statins. Importantly, based on observations, it has been proven that statin use is associated with skeletal muscle damage, and these changes are presented even in asymptomatic patients treated with statins (195).

4.3.9 Estimation of risk in COQ2 *gene* rs6818847 & rs6535454 SNPs in regarding to the lab. Parameters:

Results of the genotypes were showed in table (3-15) which were indicated that the mutation in the polymorphism of rs 6818847 SNP the COQ10 & HDL were statistically significantly different in the homozygous wild genotype (AA) ($p < 0.05$). (OR = 1.237 & 0.951; 95% confidence interval = (1.11-1.38, 0.912-0.992) , respectively.

While, In the genotype of the polymorphism of COQ2 SNP gene rs 6535454 was showed a statistically significantly different in the homozygous wild genotype (CC) ($p < 0.05$). (OR = 1.144 ; 95% confidence interval = (1.061-1.233) as shown in table (3-16). Myopathy has proven to be a difficult disease to understand and unfortunately all previous genetic links have not been replicated in larger studies. All variants known to be pathogenic or presented as of uncertain significance were analysed.

4.4. Conclusion

According to the findings, it is possible to draw the following conclusions:

1. COQ2 gene was highly polymorphic and detected with different genotypes and variable frequencies in Iraqi statin taking patients.
2. For the SNP (rs6818847), the mutant genotype (AA) was more prevalent than the other genotypes (CC and CA), and for the SNP (rs6535454), the wild genotype (AA) was more prevalent than the other genotypes (GG and AG).
3. There was a strong correlation between the COQ2 gene found in Iraqi patients using statins and lower CoQ10 serum levels, indicating that COQ2 genotypes have an effect on this parameter's levels and may therefore affect how well atorvastatin works.
4. The homogeneous mutant genotypes and heterogeneous genotypes of the COQ2 gene are connected with significantly elevated serum creatine kinase levels.

4.5 Recommendations and future directions

1. To completely identify the impact of COQ2 genetic variant on Atorvastatin side effects, increase sample size, and use case-control studies rather than cross-sectional observational research in Iraqi hyperlipidemic patients using Atorvastatin.
2. Research other COQ2 gene polymorphisms in nearby subjects to build an agentic map and identify the root of inter-individual variation in atorvastatin response.

3. More research may be necessary to determine whether genetic variations in other enzymes involved in the metabolism of atorvastatin contribute to individual variability in atorvastatin responsiveness.

4. Research the effects of Coenzyme Q10 supplementation, a straightforward and alluring treatment, to see if it can completely or partially alleviate statin-induced myalgia in symptomatic patients.

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APPENDIX

Questionnaire for Patients taking statin therapy

Demographic characterization

الاسم:
رقم الهاتف:
الوزن:
الطول:

Parameters	Variable	Notes
Age		
Gender		
Smoking	Yes No	
BMI		
Education	Primary Secondary Collage	
Type of myopathy	Myalgia Weakness Muscle cramps	
Duration of treatment of statin		
Other Drug side effects		
Other diseases		
Other medication		

Biochemical Parameters

Parameters	Results
Coenzyme Q10	
Renal function test	Creatinine:
Lipid profile	Cholesterol: Triglyceride: HDL-Cholesterol: HDL-Cholesterol:
Creatinine kinase	
TSH	
Results of Genotyping	

النتائج: كشفت النتائج التي تم الحصول عليها من هذه الدراسه عن أنماط وراثية متعددة لجين COQ2 خاصة COQ2 (A> G) (rs6535454) و COQ2 (C> A) (rs6818847)، والتي تشمل النمط الجيني متماثل الزيجه (AA)، النمط الجيني متماثل الزيجه الطافر (GG)) والنمط الجيني متغاير الزيجه (AG) لـ COQ2 (A> G) (rs6535454)) بينما تم اكتشاف النمط الجيني متماثل الزيجه (CC) ، النمط الجيني متماثل الزيجه الطافر(AA) والنمط الوراثي متغاير الزيجه (CA) لـ COQ2 (C> A) (rs6818847) للمرضى المشاركين في هذه الدراسة الذين يتناولون الستاتين. فيما يتعلق بمستوى الإنزيم المساعد Q10 وكرياتين كيناز في المصل، أظهرت الدراسة الحالية ارتباطًا معنويًا ($p<0.05$) بين SNPs المدروسة لجين COQ2 ومستوى CoQ10 في المصل، في حين أن الارتباط غير المعنوي ($p> 0.05$) بين SNPs المدروسه لجين COQ2 ومستوى انزيم الكرياتين كيناز في الدم في المرضى الذين يتناولون الستاتين المشمولون في الدراسة.

الاستنتاجات: استنتجت الدراسه وجود تأثير كبير لتعدد الأشكال الشائعة (rs6535454) و (rs6818847) داخل جين COQ2 مما يؤدي لخطر تطور المشاكل العضليه المرتبطة بعلاج الاتورفاستاتين عند مرضى ارتفاع الدهون في الدم في محافظة كربلاء المقدسة.

الخلاصة

الخلفية الدراسية: يعد الجين COQ2، المشفر 4-هيدروكسي بنزو-أتي-بوليبرينيل ترانسفيراز (الإنزيم المساعد Q2)، من العوامل التي يحتمل أن تؤثر في تحمل علاج الستاتيين ، حيث يشارك هذا الإنزيم في التكوين الحيوي للأنزيم المساعد (CoQ10)، و الذي يؤدي النقص الناتج بعقار الستاتين في ظهور أعراض مرض العضلات المرتبطة بالستاتين (SAMS). وبالتالي، فإن تعدد الأشكال في جين COQ2 قد يفسر قابلية الستاتين في تحفيز الاعتلال العضلي. يصنف الاعتلال عضلي كأحد الآثار الجانبية الرئيسية للعقاقير المخفضة للكوليسترول و التي تؤدي إلى عدم التحمليه لعقار الستاتين مما يؤدي للتوقف عن تناوله.

هدف الدراسة: هدفت هذه الدراسة للكشف عن تعدد الأشكال الجيني لجين COQ2 وخاصة COQ2 (A> G) (rs6535454) و COQ2 (C> A) (rs6818847) ، والتي تشارك في التكوين الحيوي لإنزيم CoQ10 في المرضى العراقيين الذين يتناولون جرعه 40 ملغ من عقار أتورفاستاتين.

المرضى والية العمل: أجريت هذه الدراسة المقطعية العرضية في مدينة الإمام الحسين الطبية بكربلاء. تمحيث اختير مائة وخمسون مريضا مستمرين في علاج الأتورفاستاتين للمشاركة في هذه الدراسة. تتراوح اعمار المرضى المشمولين في هذه الدراسة بين (30-65) سنه والذين يتعاطون أقراص أتورفاستاتين (40 ملغ) مرة واحدة يوميًا. سحبت عينات الدم من المرضى الذين وقعوا الموافقة لإجراء الاختبارات الجينية، وتم استخدامها لقياس مستوى انزيم Q10 في الدم، ومستوى الدهون (الكوليستيرول- الدهون الثلاثيه –البروتين الدهني منخفض الكثافه – البروتين الدهني عالي الكثافه) ، واختبار وظائف الكلى (الكرياتينين) ، وانزيم الكرياتينين كيناز، والهرمون المحفز للغدة الدرقية، استخدم في هذه الدراسة نظام التضخيم الحراري لتفاعل البوليمرات التسلسلي (ARMS PCR) للكشف عن COQ2 (A> G) (rs6535454) و COQ2 (C> A) (rs6818847) .

جامعة كربلاء
كلية الصيدلة
فرع الادوية والسموم

تأثيرتعدد الأشكال الجينية (COQ2) على حدوث الاعتلال العضلي لدى المرضى المعالجين بلأتورفاستاتين في محافظة كربلاء

رسالة
مقدمة الى مجلس كلية الصيدلة / جامعة كربلاء
كجزء من متطلبات نيل درجة الماجستير في علم الادوية والسموم

كتبت بواسطة
هاله يونس كاظم
بكالوريوس صيدلة (جامعة كربلاء -2017)

بإشراف
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2022 ميلادي **1443 هجري**