The effect of COQ2 gene polymorphism (C>A) (rs6818847) on the occurrence of myopathy in statin-treated patients

Hala Y. Kadhom[1], **Mazin H. Ouda**[2]

Abstract

Hydroxymethylglutaryl-CoA (HMG-CoA) reductase inhibitors (statins) can cause skeletal muscle toxicity; the risk of toxicity is elevated by drug interactions and pharmacogenetic factors that increase the concentration of statins in plasma. The aims of this study were to detect the genetic polymorphism of COQ2 gene particularly COQ2 (C>A) (rs6818847) that involved in biosynthesis of CoQ10 enzyme in Iraqi patients treated with 40mg atorvastatin. The study comprised 150 hyperlipidemic patients taking 40 mg atorvastatin once a day, in whom CoQ10, CK, lipid profile, TSH and renal function test were all evaluated. The Amplification Refractory Mutation System Polymerase Chain Reaction (ARMS PCR) was used in this study to identify COQ2 (C>A) (rs6818847). Results finding in this study that, COQ2 (C>A) (rs6818847), has been found to have several genotypes, all of which have been found in statin-taking research participants. The current investigation revealed a significant ($p<0.05$) connection between the studied SNP of COQ2 gene and serum CoQ10 level. Although the 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 conclusion, there is a significant impact of polymorphism (rs6818847) within the COQ2 gene in Iraqi dyslipidemic individuals treated with high doses of atorvastatin; this poses a danger of developing myopathy connected with the use of atorvastatin.

KeyWords: Myopathy, Statin, COQ2 gene, CoQ10.

DOI Number: 10.14704/nq.2022.20.8.NQ44708 **NeuroQuantology 2022; 20(8): 6832-6839**

[1] Department of Pharmacology and Toxicology, College of Pharmacy, University of Kerbala, Kerbala, Iraq
[2] Department of Pharmacology and Toxicology, College of Pharmacy, University of Kerbala, Kerbala, Iraq

Introduction

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 (1). 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) (2), 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 (3). 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. 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 (4), 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 (5).

Material and method

The Imam Al-Hussein Medical City/Cardiology Center and Al-zahraa Center hosted this prospective clinical study from October 2021 to February 2022 with participants from the University of Kerbala's College of Pharmacy and Kerbala, Iraq's outpatient clinic. 150 people who were taking the statin medicine atorvastatin participated in the study.

Each participant in the trial, who ranged in age from 30 to 65, had been using atorvastatin 40 mg for at least six months. This study was not open to patients with hypothyroidism, recent surgery, severe renal failure, hepatic failure, recurrent infection, several comorbidities, low body mass index, and those taking a number of medications.

Both the Kerbala Health Directorate and the college of pharmacy at the University of Kerbala's ethical research committee approved the study's protocol. After being informed of the study's purpose and nature, each patient and the imam of Al-Hussain Medical City gave their approval.

Blood samples were gathered from eligible patients after getting their consent. All trial participants, who had fasted overnight, had their 8 mL of venous blood drawn in the morning. After placing 3 ml in an EDTA tube for genetic testing and immediately inspecting the findings to obtain extremely pure DNA, 5 ml was added to the gel tube for the serum analysis. Serum was aspirated from the blood after it had been centrifuged at 3000 rpm for 10 minutes and utilized for analysis. The biochemical parameters that were measured for all patients were creatinine kinase (CK), Coenzyme Q10 (COQ10), renal function test (creatine), thyroid stimulating hormone (TSH), and lipid profile (total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL), and high density lipoprotein (HDL)). According to the manufacturer's instructions, genomic DNA was extracted from each blood sample using a genomic DNA extraction kit for blood (Favorgen, China). Before usage, the DNA was kept at -200C. Traditional genotyping techniques were used to genotype the COQ2 gene's SNP (rs6818847) using the Accupower® PCR PreMix -Bioneer Kit, Korea. The COQ2 gene-specific primers were used in the PCR process. Based on the NCBI database, all gene information, sequence details, and SNP information were retrieved. Using specialist software, primer designs were produced. In all statistical studies, values with $P< 0.05$ and $P <0.01$, respectively, are regarded as significant and highly significant. 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.

Results

The age categories for the study's patient

population's demographics revealed that the age group (48-65 years) is the one with the highest percentage of participants. In addition, BMI was the largest percentage among the group of overweight people, as can be seen in the table below, where females made up 54%more than males.

Table (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
Data Presented by numbers and percentage			

3.1. Genotyping of Gene (rs6818847)

The results of gene polymorphism rs6818847 was a clear band with a molecular size 120 bps. (Figure 1) The size of amplicon was determined by compare with DNA ladder 100 - 1000 bp.

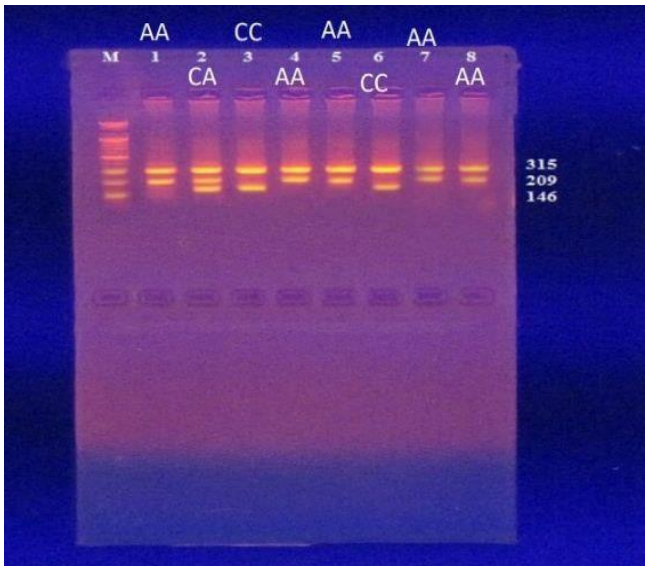

Figure (1): Genotyping of gene polymorphism rs6818847.

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).

Table (2): 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 the SNP with rs6818847 in the tested population were shown in figure (2). 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 x2 for rs6818847 gene 69.987, P < 0.001} and therefore it was statistically significant table (3).

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

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

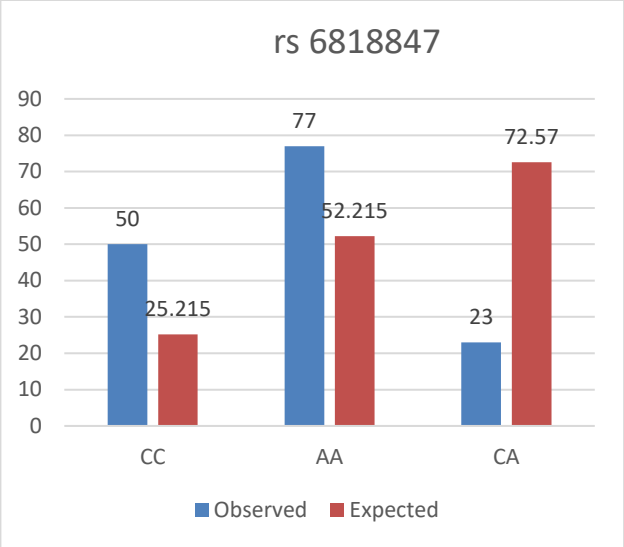

Figure (2): Observed (Obs.) vs expected (Exp.) genotype frequencies % of rs6818847 gene among individuals' sample.

3.1.1. Relationship between demographic characteristics and rs6818847 SNP.

To show the difference between demographic characteristics (mean) and rs 6818847 SNP (table 4), 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 (4): 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

3.1.2. 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 rs 6818847 SNP groups (table 5), by comparing the mean using a one-way ANOVA test. A statistically significant difference was found among mean of COQ10 between rs 6818847 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 rs 6818847 SNP groups, ($p > 0.05$).

Table (5): difference between Lab finding mean in rs 6818847 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				

3.2. Estimation of risk

3.2.1. 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 in the myopathic patients treated with statin Table (6).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 (6): The odds ratios of COQ2 gene rs 6818847 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]	-
Results are presented [S]; Significant, [NS]; Non significant, OR: Odds Ratio, CI; Confidence Interval, 1[a]; reference category			

Discussion

Myopathy, which includes myalgia (muscle pains without an elevation in creatinine kinase) and myositis, is the most common side effect of statin drugs (muscle symptoms with increased creatinine kinase level). Rarely, it can get so bad that rhabdomyolysis develops, which can be fatal (muscle symptoms with marked elevation of creatinine kinase and myoglobinuria). The incidence of statins-induced myopathy reported in past investigations ranged from 5% to 20%, depending on the diagnostic criteria for myopathy and the type of 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 (6).

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 (7).

Statins lower CoQ10 levels in two ways: first, by directly blocking the mevalonate pathway enzyme HMG-CoA reductase; second, by decreasing CoQ10 transport capacity as a result of lower LDL levels (8).

In this study CoQ10 serum level was measured for all participants, 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 did not rise to be statically significant. It is, therefore, not surprising that, starting with Folkers et al. of (1985) (9). 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,(10) 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 (11), 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 (12), The sole study with unfavorable findings included 12 healthy patients and shown that the blood CoQ10 level remained unchanged after treatment.

The enzyme known as creatine kinase (CK) is responsible for catalyzing the conversion of phosphocreatine and adenosine diphosphate from creatine and adenosine triphosphate (ATP) (ADP). The phosphocreatine produced by this reaction is used to provide the brain, skeletal muscles, and the heart, among other tissues and cells, with the significant amounts of ATP they need. Unbalanced CK levels can result from a variety of illnesses, including rhabdomyolysis, heart disease, and renal disease. or even certain drugs (13).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 (14). 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 (15). Because hypothyroidism can produce generalized muscular enlargement, along with stiffness, weakness, and severe muscle cramps, serum CK levels are typically high (16).

The main finding of this research is that atorvastatin affects 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) (17) who reported that an initial effect of intensive atorvastatin therapy on HDL-C and TG levels is different from a long-term effect: they saw an acute decrease in HDL-C and an acute increase in TG levels. There is currently weak data supporting the immediate lipid-lowering effects of statins in these patients. It was determined that the immediate acute effect of intensive atorvastatin therapy, which is started when patients with ACS are admitted, varies from the long-term effects of statin medication (18,19).The COQ2 gene, which is responsible for the enzyme 4-hydroxybenzoate-polyprenyltransferase, is one of the candidates that could have an impact on how well statin therapy is tolerated (coenzyme Q2). The generation of coenzyme Q10 (CoQ10), whose depletion is believed to be a contributing factor to the start of statin-related muscle adverse effects, is aided by this enzyme. Therefore, COQ2 gene polymorphisms may provide an explanation for why certain individuals are more vulnerable to the statins' muscular adverse effects (20).The allele and genotype frequencies of rs 6818847 was shown in table (3) 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 rs 6818847 polymorphisms were segregated based on the presence and absence of statin-induced myopathy. The genotype frequency of the rs 6818847 polymorphism was 33.3% for homozygous wild genotype, 15.3% for heterozygous individuals, and about 51.4% 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 .Based on creatine kinase level in table (5) 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 this SNP as shown in table (5) 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 (21).

As shown in table (5) 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.

Results of the genotypes were showed in table (6) which were indicated that the mutation in the polymorphism of rs 6818847 SNP the COQ10 were statistically significantly different in the homozygous wild genotype (AA) ($p < 0.05$). (OR = 1.237; 95% confidence interval = (1.11-1.38).

Conclusion

Strong correlations between the CoQ2 gene and decreased CoQ10 serum levels were observed in Iraqi patients using statins, suggesting that COQ2 genotypes have an impact on this parameter's levels and may consequently impair how well atorvastatin functions. The serum creatine kinase levels of both homogeneous mutant genotypes and heterogeneous genotypes of the COQ2 gene are markedly raised. In conclusion, we advise lowering the dose of atorvastatin or switching to another lipid-lowering medication that has less of an impact on muscles in order to prevent the development of myopathy with statin use

References

1. Farmer JA. The effect of statins on skeletal muscle function: The stomp trial. Curr Atheroscler Rep. 2013;15(8):1-2.
2. Evans M, Rees A. Effects of HMG-CoA reductase inhibitors on skeletal muscle: Are all statins the same? Drug Saf. 2002;25(9):649-63.
3. Bairey-merz CN, Grundy SM, Cleeman JI. Acc / Aha / Nhlbi Clinical Advisory on Statins. 2002;40(3):567-72.
4. Ucar M, Mjörndal T, Dahlqvist R. HMG-CoA reductase inhibitors and myotoxicity. Drug Saf. 2000;22(6):441-57.
5. Parker BA, Thompson PD. Effect of statins on skeletal muscle: Exercise, myopathy, and muscle outcomes. Exerc Sport Sci Rev. 2012;40(4):188-94.
6. Iwere RB, Hewitt J. Myopathy in older people receiving statin therapy: A systematic review and

meta-analysis. Br J Clin Pharmacol. 2015;80(3):363–71.

7. Hargreaves I, Heaton RA, Mantle D. Disorders of human coenzyme q10 metabolism: An overview. Int J Mol Sci. 2020;21(18):1–13.
8. Deichmann R, Lavie C, Andrews S. Coenzyme Q10 and statin-induced mitochondrial dysfunction. Ochsner J. 2010;10(1):16–21.
9. Folkers K, Wolaniuk J, Simonsen R, Morishita M, Vadhanavikit S. Biochemical rationale and the cardiac response of patients with muscle disease to therapy with coenzyme Q10. Proc Natl Acad Sci U S A. 1985;82(13):4513–6.
10. Mortensen SA, Leth A, Agner E, Ronde M. Dose-related decrease of serum coenzyme Q10 during treatment with HMG-CoA reductase inhibitors. Mol Aspects Med. 1997;18(SUPPL.):137–44.
11. Rundek T, Naini A, Sacco R, Coates K, DiMauro S. Atorvastatin decreases the coenzyme Q10 level in the blood of patients at risk for cardiovascular disease and stroke. Arch Neurol. 2004;61(6):889–92.
12. Bleske BE, Willis RA, Anthony M, Casselberry N, Datwani M, Uhley VE, et al. The effect of pravastatin and atorvastatin on coenzyme Q10. Am Heart J. 2001;142(2):13A.
13. Chanson JB, Dakayi C, Lannes B, Echaniz-Laguna A. Benign acute myositis in an adult patient. BMJ Case Rep. 2018;2018.
14. Moghadam-Kia S, Oddis C V., Aggarwal R. Approach to asymptomatic creatine kinase elevation. Cleve Clin J Med. 2016;83(1):37–42
15. Rush J, Danzi S, Klein I. Role of thyroid disease in the development of statin-induced myopathy. Endocrinologist. 2006;16(5):279–85.
16. Bar SL, Holmes DT, Frohlich J. Asymptomatic hypothyroidism and statin-induced myopathy. Can Fam Physician. 2007;53(3):428–31.
17. Vondrakova, D., Ostadal, P. & Kruger, A. Immediate effect of intensive atorvastatin therapy on lipid parameters in patients with acute coronary syndrome. Lipids Health Dis 9, 71 (2010). 2010. 2010.
18. Mariam Maqsood, Saleha Sadeeqa, Maqsood Ahmad, Hafsa Afzal., Efficacy and safety of atorvastatin and rosuvastatin in ischemic heart disease patients: A prospective study. Tropical Journal of Pharmaceutical Research July 2019 and 1533-1538, 18 (7):. 2019. 2019.
19. Nichols GA, Koro CE. Does statin therapy initiation increase the risk for myopathy? An observational study of 32,225 diabetic and nondiabetic patients. Clin Ther 2007 and 29(8):1761-1770. 2007. 2007.
20. Hopewell JC, Reith C, Armitage J. Pharmacogenomics of statin therapy: Any new insights in efficacy or safety? Curr Opin Lipidol. 2014;25(6):438–45.
21. Fallah M, Askari G, Soleimani A, Feizi A, Asemi Z. Clinical trial of the effects of coenzyme q10 supplementation on biomarkers of inflammation and oxidative stress in diabetic hemodialysis patients. Int J Prev Med. 2019;10(1).