A STUDY OF EFFECTS OF ADDITION OF COENZYME Q 10 TO HIGH DOSE STATINS IN PATIENTS OF CORONARY ARTERY DISEASE WITH SPECIAL REFERENCE TO OXIDATIVE BALANCE

Malleswara Rao Dangeti, Siva Krishna Katkam, Raghu Kishore Galla, Ravi Kiran, Indrani Garre

ABSTRACT:

Background: *Oxidative stress is one of the most potent inductors of endothelial dysfunction and is involved at all stages of atherosclerotic plaque evolution. Statins are 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors and are potent inhibitors of cholesterol biosynthesis. In clinical trials, statins are beneficial in the primary and secondary prevention of coronary heart disease. Statins also possess direct free radical scavenging activity. However, the prooxidant effect of statins has also been reported as statins block the mevalonate pathway and the synthesis coenzyme Q10.This Additional Coenzyme Q10 depletion by statins in patients with coronary artery disease (CAD) may be a critical issue as it may reduce absolute benefits of statins.*

Objectives: *The purpose of this study was to investigate the effects of high dose statins on plasma Malondialdehyde (MDA) levels and plasma glutathione levels in CAD patients who underwent recent PCI and to study whether addition of coenzyme Q10 (100 mg/d) has any additional effect on plasma Malondialdehyde (MDA) levels and plasma glutathione levels in patients already receiving high dose statin therapy.*

Methods: *Twenty-one consecutive patients who underwent percutaneous transluminal coronary angioplasty (PTCA)in Department of Cardiology at our institute were studied. The cases (n = 21) were given high dose statins for first 1 week and then coenzyme Q10 (100 md /day) is added for next 1 week.Plasma Malondialdehyde(MDA) levels and plasma glutathione levels were analyzed at the time of admission before giving statins and at the end of 1 week of statin therapy and again after 1 week of Co-Q therapy.*

Results: *Our results indicate that a relation exists between high plasma Malondialdehyde (MDA) levels and low plasma glutathione levels with coronary artery disease. High dose statins decrease MDA levels and increase plasma glutathione levels, even though they decrease coenzyme q levels in the body. It was also shown that addition of Coenzyme Q10 at 100 mg/d enhances plasma glutathione levels and decreases plasma MDA level still further in patients who have CAD, already receiving high dose statin therapy.*

Conclusions: *Addition of Coenzyme Q10 at 100 mg/d has an additive effect with high dose statins in decreasing oxidative stress. Particularly in light of the excellent tolerance and affordability of this natural physiological compound, supplemental Coenzyme Q10 may emerge as an attractive option in future, and merits evaluation in additional large studies.*

Keywords: *Co-Enzyme Q10, Statins, Oxidative Stress, Coronary Artery Disease*

Article received on 01 JAN2017, published on 31JAN 2017.

Malleswara Rao Dangeti[1], Siva Krishna Katkam[2], Raghu Kishore Galla[1], Ravi Kiran[1],Indrani Garre[3]
[1]Senior Resident, Department of Cardiology, NIMS, India
[2]Student ,Department of Clinical Pharmacology, NIMS ,India
[3]PhDStudent ,Department of Cardiology, NIMS, India
Corresponding author: Malleswara Rao Dangeti
Email: ramakrishnaji83@gmail.com

INTRODUCTION:

Coronary artery disease (CAD) is one of the leading causes of morbidity and mortality in developed countries and is emerging as an epidemic in developing countries [1]. It has been known that there was a relation between the levels of oxidative stress and the severity of the CAD [2]. Published studies have demonstrated that DNA damage that is incurred by oxidative stress contributes significantly to the development and the progression of atherosclerosis [3, 4].

Lipid peroxidation, which is mediated by free radicals, is thought to be the major mechanism of cell damage. Free radicals are formed in both physiological and pathological conditions in mammalian tissues [5,6]. The recent studies showed that Malondialdehyde (MDA) is an important marker of lipid peroxidation, hence it can be used as an indicator of the oxidative stress in tissue and cells. Previous data has shown that progression of atherosclerosis is correlated with oxidative stress and can be followed up by MDA measurements [7].

Cells have an efficient antioxidant defence system, mainly composed of the enzymes such as superoxide dismutase (SOD) and glutathione peroxidise (GPX).

Coenzyme Q10 (CoQ10) is an endogenously synthesized and diet-supplied lipid-soluble cofactor that functions in the mitochondrial inner membrane to transfer electrons from complexes I and II to complex III. In addition, its redox activity enables Coenzyme Q10 to act as a membrane antioxidant [8].

Statins are 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors that reduce the risks of acute cardiac events and death [9-1].Although widely used for their lipid-lowering effects, statins are also reported to have anti-inflammatory effects [12-14]. Statins also possess direct free radical scavenging activity. However, the prooxidant effect of statins has also been reported as statin block the mevalonate pathway and the synthesis CoQ_{10} [15-17]. Because of this additional Coenzyme Q10 depletion via statins in patients with CAD, it is difficult to predict the effect of high dose statins on lipid peroxidation and levels of antioxidant enzymes. For this reason, we determined serum MDA levels as the index of lipid peroxidation, the serum GPX as antioxidant enzymes in patients with CAD and analyzed how they respond to high dose statins alone and with a combination of high dose statin and CoQ10.

MATERIAL AND METHODS:

The study was conducted in the Department of Cardiology at our institute Hyderabad. The study started on October 2, 2015and ended on March 21, 2016. Twenty-one consecutive patients who admitted with a diagnosis of chronic stable angina or acute coronary syndromes who underwent percutaneous transluminal coronary angioplasty (PTCA) were recruited. The study was approved by the institutional review board. An informed written consent was obtained from the participants before recruiting them in the study. Major exclusion criteria included the use of any antioxidant medication within 30 days of the baseline visit, subjects with liver or renal diseases, or who have contraindication for high dose statins. Those patients who had acute or chronic inflammatory disorders, immunological diseases, and current neoplastic disease are also excluded.

Baseline data were collected regarding age, sex, and conventional CAD risk factors (diabetes mellitus, hypertension, and smoking). Diagnosis and indication for PCI were recorded. All patients were subjected to undergo routine investigations including complete blood counts, blood sugar, liver function tests, lipid profile, blood urea, serum creatinine, and serum electrolytes. Electrocardiography (ECG) and transthoracic echocardiography [TTE] were performed for all patients. Additional 5 ml of venous blood was collected into EDTA bottles at the time of confirmed diagnosis before starting treatment on admission to the hospital for determination of plasma Malondialdehyde (MDA) levels and plasma glutathione levels which served as baseline levels in CAD patients.

Eligible patients were advised to take high dose statins (Rosuvastatin 40 mg or Atorvastatin 80 mg) daily once and asked to return within one week. Serum samples were again obtained for determination of plasma Malondialdehyde (MDA) levels and plasma glutathione levels at the end of 1week.Then they are advised to take Coenzyme Q 10 100 mg daily once for 1 week and asked to come again after 1 week. Serum samples were once again taken for estimation of plasma Malondialdehyde (MDA) levels and plasma glutathione levels.All possible adverse effects were monitored from the start of the study at every visit. Compliance was tracked through medication logs. Use of concomitant medications was tracked at all visits.

For MDA estimation, Venous blood sample (5 ml) was collected into EDTA bottles.To 1.0 ml of plasma (test/control) standard (collected in a test tube), 1.0 ml of 30% trichloroacetic acid (TCA), 200 µl of 1% thiobarbituric acid (TBA) were added and vortexed for 30 sec. The test tubes were incubated at 85°C in a water bath for 1 hour. After incubation, the test tubes were immediately cooled by placing them in ice-cold water for 10 min, followed by centrifugation at 10,000 rpm for 5 min. 1.0 ml of clear supernatant was collected and transferred into a cuvette and the absorbance was read at 540 nm against blank using UV-Visible Spectrophotometer (UV-1601, Schimatzu). The concentration of MDA was read from standard calibration curve plotted using TEP. The results were expressed in nanomoles of MDA per ml of plasma. For

estimation of plasma GSH activity levels, about 6.0mls of venous blood were obtained into EDTA bottles. To, 80 µl of plasma (test/control) /standard (collected in a test tube), 300 µl of dilution buffer, 100 µl of DTNB reagent, 1.6 ml of Acetonitrile were added and mixed well. Then the tubes were incubated at 37°C for 5 min followed by centrifugation at 3000 rpm for 5 min. 1.0 ml of clear supernatant was collected and transferred into a cuvette and the absorbance was measured at 412 nm against to blank by using UV-Visible Spectrophotometer (UV-1601, Schimatzu) (Ellman, 1959).

Plasma Malondialdehyde (MDA) levels and Plasma Glutathione levels were analyzed and compared between cases and controls. Controls are those subjects who have normal coronaries on diagnostic angiography. A total of 34 subjects were taken as control. This study was conducted according to good clinical practice guidelines. All analyses were performed by the independent statistician after the study was terminated.

RESULTS:

Participants were enrolled in this trial between October 2, 2015, and March 21, 2016. A total of 21 patients enrolled into the study (Mean age 57.47± 8.45). Baseline characteristics are shown in Table 1.

Table1.Baseline Characteristics (n=21)

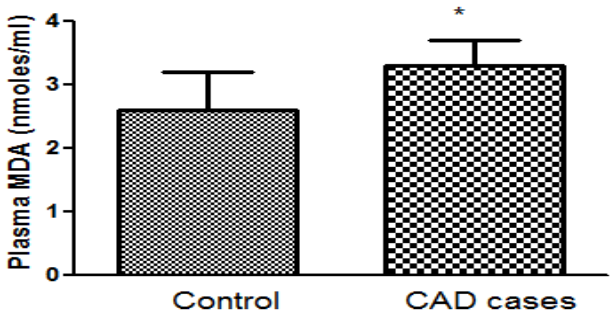
Number of patients		21
Age, yrs		57.47 ± 8.45
Male, %		61.9
Comorbidities, %	Hypertension	76.19
	Diabetes	52.38
	Smoking history	52.38
Clinical presentation, %	Stable angina	42.86
	Unstable angina	33.33
	Myocardial infarction	23.81
NYHA class, %	NYHA class I, II	80.95
	NYHA class III, IV	19.05

Of these 21patients, 13(61.9%) were males and 8(38.1%) were females. Histories of systemic arterial hypertension were present in 16 (76.19%), diabetes mellitus in 11(52.38%), and smoking in 11(52.38%). Most common indication for PCI was chronic stable angina (42.85%) followed by Myocardial Infarction (33.33 %). Rest of the patients had unstable angina (23.80%). The majority of patients (81%) were in NYHA functional class I and II. Mean Ejection fraction at time of admission as calculated by biplane method of disks (modified Simpson's rule) was 53 ±6 %.

Baseline mean LDL cholesterol level at time of admission was 98.10± 41.99 mg per decilitre. As expected, mean LDL cholesterol level decreased significantly after 15 days with high dose of statins to 62.67± 11.29(95% CI: 17.86 to 52.99,p<0.001).Mean baseline triglyceride levels decreased from 120.24 ± 42.57 mg per decilitre to 85.38 ±13.84 mg per decilitre (95% CI: 15.12 to 54.60, p <0.01).

Plasma MDA levels were found to be significantly elevated in CAD cases (p<0.001) compared to controls (Figure 1).

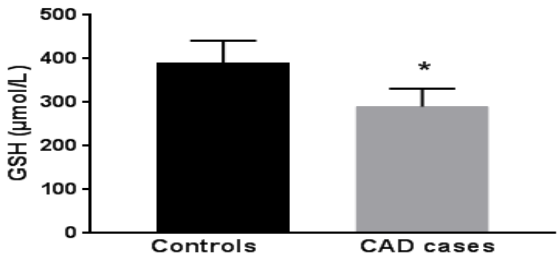
Fig 1: Plasma Malondialdehyde (MDA) levels in healthy controls and CAD patients. Values are expressed as mean ± SD. *p <0.05 vs control.



The values in CAD cases and controls are 3.21 ± 0.63 nmoles/ ml and 2.6±0.6 nmoles/ml, respectively. Mean MDA levels in patients presented with chronic stable angina, unstable angina, and myocardial infarction were 2.77± 0.45, 3.19±0.60, and 3.82± 0.30nmoles/ml, respectively.

Levels of total glutathione were estimated in plasma of patients and controls. The glutathione levels were found to be significantly low in cases compared to controls (390 ± 51 vs. 290 ± 41 µM/L) as shown in Fig 2.

Fig 2: Plasma Glutathione levels in CAD cases and healthy controls. Values are expressed as mean ± SD. *p<0.001 vs control



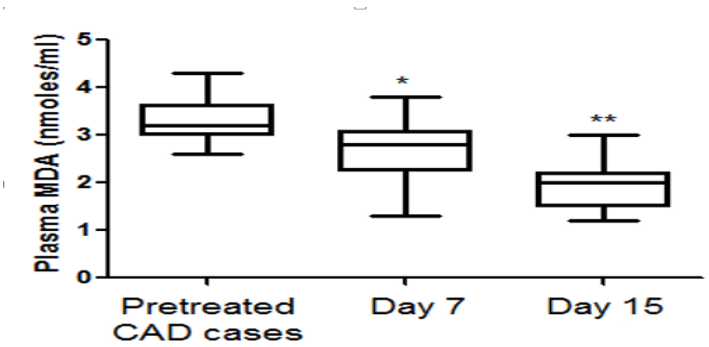
Mean total glutathione levels in patients presented with chronic stable angina, unstable angina, and myocardial infarction was 322.11± 56.53,308± 45.65, and 261.71± 34 .76, respectively.

Next, we determined the plasma MDA and GSH levels in CAD patients on day 7 and day 15. As shown in Table 2, Fig 3.

Table 2: Plasma MDA levels and plasma glutathione levels at the time of admission, at 7th day and at 15th day

	Baseline	After 1 week of statin therapy	After 1 week of CoQ10 therapy
Plasma MDA levels (nM/ml)	3.219 ±0.6325	2.619 ± 0.6486	1.9852 ± 0.4396
Plasma glutathione levels (µM/L)	298.619 ± 48.9167	346.6667 ± 62.3629	376.4286 ± 110.2228

Fig 3: Plasma malonaldehyde (MDA) levels in CAD cases (pretreatment) and on day 7 and 15. Values are expressed as mean ± SD *p<0.01



Plasma MDA levels were significantly decreased with high dose statin therapy compared to baseline values. Plasma MDA levels were significantly decreased with the addition of Co-Q compared to day 7 values. Similarly, there was a significant increase in GSH levels by statins compared to baseline as shown in Table 3.

Table 3: Effect of statins on plasma MDA levels and plasma glutathione levels.

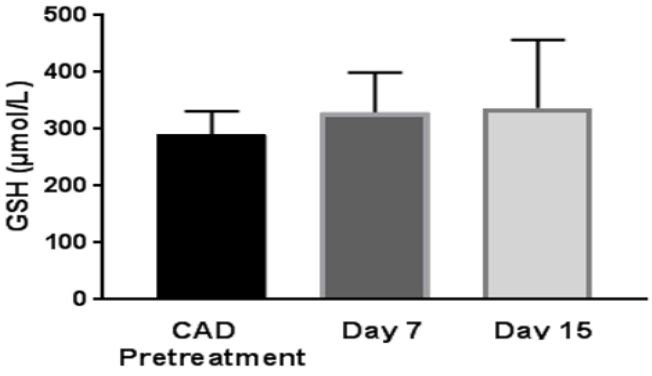
	Baseline	After 1 week of statin therapy	P value
Plasma MDA levels(nM/ml)	3.219 ±0.6325	2.619 ± 0.6486	0.0011
Plasma glutathione levels (µM/L)	298.619 ± 48.9167	346.6667 ± 62.3629	0.0083

There was an increase in GSH levels with the addition of CoQ10, However, that was not statistically significant (shown in Table 4, Fig 4).

Table 4: Effect of CoQ10 on plasma MDA levels and plasma glutathione levels

	After 1 week of statin therapy	After 1 week of CoQ10 therapy	pvalue
Plasma MDA levels(nM/ml)	2.619 ± 0.6486	1.9852 ± 0.4396	0.0001
Plasma glutathione levels (µM/L)	346.6667 ± 62.3629	376.4286 ± 110.2228	0.1486

Fig 4: Plasma glutathione levels in CAD cases (pretreatment) and on day 7 and 15. Values are expressed as mean ± SD.



DISCUSSION:

In this study, we demonstrated that Plasma MDA levels were found to be significantly elevated in CAD cases ($p<0.001$) compared to controls having normal coronaries on diagnostic angiography. Plasma MDA levels were significantly decreased with high dose statin therapy which were further decreased with addition of CoQ10 (100 mg/day). Plasma GSH activity was low in CAD cases compared to cohort of controls having normal coronaries on diagnostic angiography. Plasma GSH activity measured on day 7 and 15, there was an increase in GSH levels as compared to pre-treated CAD cases.

High oxidative stress and chronic inflammation are major contributors to the pathogenesis of CAD. Antioxidant enzymes such as SOD, CAT, and GPx are the first line of defence against reactive oxygen species [18] and a decrease in their activities contributes to the elevated oxidative stress in CAD patients [19]. Antioxidant enzymes (SOD, GPX) are compounds that dispose, scavenge, and suppress the formation of free radicals and oppose their actions. Plasma GSH activity, an oxidative stress inducible enzyme, plays a significant role in peroxyl scavenging mechanism, and in maintaining functional integration of the cell membranes. Alteration in the oxidant - antioxidant profile is known to occur in CAD [23,24]. Observation studies proposed that oxidative dysfunction occurs in the pathogenesis of CAD and are consistent with the process of lipid peroxidation and oxidative DNA damage. Hence, CAD is considered to be a chronic inflammation status [25].

In the present study, the lipid peroxidation product i.e. MDA levels have been increased significantly in plasma of the patients with coronary artery disease as compared to the controls. In our study, Statins decreased plasma MDA levels by 18 % and addition of coenzyme Q10 at 100 mg/d further decreased plasma MDA levels by 24%. Belch et al. showed that progression of atherosclerosis is correlated with oxidative stress and can be followed up by MDA measurements [27].Results of the studies of Pezeshkianet al. showed that MDA levels increased significantly in heart diseases [28]. Some other investigates have also reported an increase of SOD, MDA and plasma GSH activity levels in patients with CAD (29).The recent studies showed that Malondialdehyde (MDA) is an important marker of lipid peroxidation and progression of atherosclerosis is correlated with oxidative stress and can be followed up by MDA measurements [30].

Coenzyme Q10 is an endogenous antioxidant. It inhibits both the initiation and the propagation of lipid and protein oxidation. Statins block the mevalonate pathway and the synthesis of both cholesterol and Coenzyme Q10 [33,34]. Additional Coenzyme Q10 depletion via statins in patients with CAD may be a critical issue and may at least theoretically have contributed to neutral outcomes of RTCs with statins in heart failure. Mabuchi et al. reported that serum levels of Coenzyme Q10 diminished during treatment with statin in patients with hypercholesterolemia [34]. The information about the association between oxidant-antioxidant balance and statin therapy is scarce.

In the present study, we have found that plasma GSH enzyme activity was significantly lower in patients with CAD than controls. We have found that statins increased plasma GSH enzyme activity by 16 %, the addition of coenzyme Q10 at 100 mg/d further increased the activity of plasma GSH activity by8.5 %.However, Lee BJ, Huang YC et al. showed that coenzyme Q10 at a dose of 150 mg/d increased the activity of SOD by 22.2% and of CAT by 4.5%, but had no effect on that of Plasma GSH activity [31]. Tiano et al. [32] administered coenzyme Q10 (300 mg/d) to patients with ischemic heart disease for 1 month, and they observed that those patients' extracellular superoxide dismutase activity and endothelium-dependent vasodilatation were improved after supplementation. Coenzyme Q10 supplementation at 300 mg/ day significantly enhanced antioxidant enzymes activities and lowered inflammation in a study by Lee BJ et al [33].

Regarding the safety of coenzyme Q10 and its combination with statin therapy in the present study, there were no clinically significant changes in the subjects' vital signs, serum chemical values, or hematological values, and there were no serious adverse events and no withdrawals due to adverse events. Thus, coenzyme Q10 at a dose of 100 mg/d is safe for co-administration with statins therapy.

Study Limitations

Our study should be viewed in the context of certain limitations. This study was a small, single-centre study at a tertiary care hospital with short follow-up duration. The small number of patients included in the study might have influenced the results. Second, we do not

have data on the changes in serum Coenzyme Q levels without which it is very difficult to comment on effect statins on Coenzyme Q levels. We lacked cohort of the control group to compare above results. Therefore, further investigations using a larger sample size are necessary to confirm the present results.

CONCLUSIONS:

In summary, our results demonstrate that addition of Coenzyme Q10 to the patients taking high dose statin therapy is safe, well tolerated and has an additive effect as an antioxidant**.**

ABBREVIATIONS:

HMG-CoA	3-hydroxy-3-methylglutaryl coenzyme A
MDA	Malonaldehyde
PTCA	percutaneous transluminal coronary angioplasty
DNA	DNA
SOD	Superoxide dismutase
PCI	Percutaneous coronary intervention
ECG	Electrocardiography
EDTA	Ethylenediaminetetraacetic acid
TBA	Thiobarbituric acid
UV	Ultraviolet
LDL	Low-density lipoprotein
CoQ10	Coenzyme Q_{10}
GSH	Glutathione
GPX	Glutathione Peroxidase
TCA	Trichloroacetic acid
TTE	Transthoracic echocardiography

REFERCENS:

1. Yusoff K. Vitamin E in cardiovascular disease: has the die been cast?. Asia Pac J ClinNutr. 2002;11:443-447.

2. Demirbag R, Yilmaz R, Kocyigit A. Relationship between DNA damage, total antioxidant capacity and coronary artery disease. Mutat Res. 2005;570:197-203.

3. Ndreassi MG, Botto N, Cocci F, Battaglia D, Antonioli E, Masetti S, Manfredi S, Colombo MG, Biagini A, Clerico A. Methylenetetrahydr of olatereductase gene C677T polymorphism, homocysteine, vitamin B12, and DNA damage in coronary artery disease. Hum Genet. 2003;112:171-177.

4. Ross R. Atherosclerosis-an inflammatory disease. N Engl J Med. 1999;340:115-26.

5. Tas F, Hansel H, Belce A, Ilvan S, Argon A, Camlica H, Topuz E. Oxidative stress in breast cancer. Med Oncol. 2005;22:11-15.

6. Priya VV, Surapaneni KM. Erythrocyte lipid peroxidation, glutathione, ascorbic acid, vitamin E, antioxidant enzymes and serum homocysteine levels in patients with coronary artery disease. Journal of Clinical and Diagnostic Research. 2008;2:1180-1185.

7. Mogadam RAP, Nemati A, Baghi AN. Serum MDA as a Diagnostic`s Biomarker in Stable Coronary Heart Disease. Research Journal of Biological Sciences. 2008;3:206-210.

8. Ernster L, Dallner G. Biochemical, physical and medical aspects of ubiquinone functions. Biochim Biophys Acta. 1995;1271:195-204.

9. Angela Park, Juliana Barrera-Ramirez, Indee Ranasinghe, Sophie Pilon, Richmond Sy, Dean Fergusson, David S. Allan. (2016) Use of Statins to Augment Progenitor Cell Function in Preclinical and Clinical Studies of Regenerative Therapy: a Systematic Review. Stem Cell Reviews and Reports 12:3, 327-339.

10.Lowie E G W Vanfleteren, Martijn A Spruit, Emiel F M Wouters, Frits M E Franssen. (2016) Management of chronic obstructive pulmonary disease beyond the lungs. The Lancet Respiratory Medicine.

11.Mario Cazzola, Paola Rogliani, JosuelOra, Maria Gabriella Matera. (2016) Treatment options for moderate to very severe chronic obstructive pulmonary disease. Expert Opinion on Pharmacotherapy 17, 977-988.

12.Meredith L. Howard, Ashley H. Vincent. (2016) Statin Effects on Exacerbation Rates, Mortality, and Inflammatory Markers in Patients with Chronic Obstructive Pulmonary Disease: A Review of

Prospective Studies. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy 36:5, 536-547.

13.Catalina Balaguer, Alejandro Peralta, Ángel Ríos, Amanda Iglesias, Josep Lluís Valera, Aina Noguera, Joan B. Soriano, Àlvar Agustí, Ernest Sala-Llinas. (2016) Effects of simvastatin in chronic obstructive pulmonary disease: Results of a pilot, randomized, placebo-controlled clinical trial. Contemporary Clinical Trials Communications 2, 91-96.

14.Jeanette P. Brown, Carlos H. Martinez. (2016) Chronic obstructive pulmonary disease comorbidities. Current Opinion in Pulmonary Medicine 22, 113-118.

15. Mortensen SA, Leth A, Agner E, Rohde M. Dose-related decrease of serum coenzyme Q10 during treatment with HMG-CoA reductase inhibitors. Mol Aspects Med 1997;18 Suppl: S137-44. Sander S, Coleman CI, Patel AA.

16.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;61:889–92.

17. De Pinieux G, Chariot P, Ammi-Said M, et al. Lipid-lowering drugs and mitochondrial function: effects of HMG-CoA reductase inhibitors on serum ubiquinone and blood lactate/pyruvate ratio. British Journal of Clinical Pharmacology. 1996;42:333–7.

18.Bahorun T, Soobrattee MA, Luximon-Ramma V, Aruoma OI: Free radicals and antioxidants in cardiovascular health and disease. Internet J Med Update. 2006, 1: 25-41.

19.Lee BJ, Lin YC, Huang YC, Ko YW, Hsia S, Lin PT: The relationship between coenzyme Q10 and oxidative stress, antioxidant enzymes activities and coronary artery disease. Scientific World Journal. 2012, 2012: 792756.

20.Priya VV, Surapaneni KM. Erythrocyte lipid peroxidation, glutathione, ascorbic acid, vitamin E, antioxidant enzymes and serum homocysteine levels in patients with coronary artery disease. Journal of Clinical and Diagnostic Research. 2008;2:1180-1185.

21. Sies H. Oxidative stress: from basic research to clinical application. Am J Med. 1991;91:31-38.

22.Cotgreave IA, Moldéus P, Orrenius S. Host biochemical defense mechanisms against prooxidant. Annu Rev Pharmacol Toxicol. 1988;28:189-212.

23.Demirbag R, Yilmaz R, Kocyigit A. Relationship between DNA damage, total antioxidant capacity and coronary artery disease. Mutat Res. 2005;570:197-203/ Andreassi MG, Botto N, Cocci F, Battaglia D, Antonioli E, Masetti S, Manfredi S, Colombo MG, Biagini A, Clerico A.

24.Methylenetetrahydr of olatereductase gene C677T polymorphism, homocysteine, vitamin B12, and DNA damage in coronary artery disease. Hum Genet. 2003;112:171-177.

25.Demirbag R, Yilmaz R, Kocyigit A. Relationship between DNA damage, total antioxidant capacity and coronary artery disease. Mutat Res. 2005;570:197-203/ Andreassi MG, Botto N, Cocci F, Battaglia D, Antonioli E, Masetti S, Manfredi S, Colombo MG, Biagini A, Clerico A. Methylenetetrahydr of olatereductase gene C677T polymorphism, homocysteine, vitamin B12, and DNA damage in coronary artery disease. Hum Genet. 2003;112:171-177.

26.Raharjo S, Sofos JN, Schmidt GR. Solid-phase acid extraction improves thiobarbituric acid methods to determine lipid oxidation. J Food Sci. 1993;58:921-924.

27.Belch JJ, Mackay IR, Hill A, Jennings P, McCollum P. Oxidative stress is present in atherosclerotic peripheral arterial disease and further increased by diabetes mellitus. Int Angiol. 1995;14:385-388.

28.Pezeshkian M, Nouri M, Zahraei M, Afrasiabi A, Abadi NA. Study of MDA, antioxidant vitamins, lipoproteins serum levels and anthropometry parameters in coronary artery disease patients. Medical Journal of Islamic Academy of Sciences. 2001;14:5-8.

29.Priya VV, Surapaneni KM. Erythrocyte lipid peroxidation, glutathione, ascorbic acid, vitamin E, antioxidant enzymes and serum homocysteine levels in

patients with coronary artery disease. Journal of Clinical and Diagnostic Research. 2008;2:1180-1185.

30. Mogadam RAP, Nemati A, Baghi AN. Serum MDA as a Diagnostic`s Biomarker in Stable Coronary Heart Disease. Research Journal of Biological Sciences. 2008; 3:206-210.

31. Lee BJ, Huang YC, Chen SJ, Lin PT: Coenzyme Q10 supplements reduce oxidative stress and increase activities of antioxidant enzymes in patients with coronary artery disease. Nutrition. 2012, 28: 250-255. 10.1016/j.nut.2011.06.004.

32. Tiano L, Belardinelli R, Carnevali P, Principi F, Seddaiu G, Littarru GP: Effect of coenzyme Q10 administration on endothelial function and extracellular superoxide dismutase in patients with ischaemic heart disease: a double-blind, randomized controlled study. Eur Heart J. 2007, 28: 2249-2255. 10.1093/eurheartj/ehm267.

33.Lee BJ, Tseng YF, Yen CH. Effects of coenzyme Q10 supplementation (300 mg/day) on antioxidation and anti-inflammation in coronary artery disease patients during statins therapy: a randomized, placebo-controlled trial. Nutr J. 2013;12:142.